

RESPIRATORY DRIVE IN A RABBIT MODEL OF PULMONARY
EMPHYSEMA

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DECLARATION

I declare that the contents of this thesis are my own work and that they have not been presented to any University other than University of Edinburgh

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ABSTRACT

This thesis describes an investigation of the respiratory drive to breathe in a rabbit model of pulmonary emphysema.

I propose that an altered respiratory drive in emphysematous rabbits may model the origin of the sensation of dyspnoea experienced by many human patients with emphysema.

Pulmonary emphysema was induced in Dutch rabbits by endotracheal administration of type IV porcine pancreatic elastase. In rats it was induced by endotracheal administration of papain.

Respiratory drive was measured as phrenic activity (phrenic slope, G and phrenic height, H), since emphysema changes the mechanical properties of the lungs and ventilation will be changed for a given respiratory drive.

Rats' and rabbits' conscious pattern of breathing (expiratory time, t_E ; inspiratory time, t_I & tidal volume, V_T) was measured barometrically to investigate the role of sensation in changed conscious pattern of breathing.

To elucidate the role of pulmonary receptors in changing the pattern of breathing and respiratory drive, the respiratory variables were measured during 3 stages: 1) preSO₂: when all pulmonary receptors were intact 2) postSO₂: when pulmonary stretch receptors (SARs) were blocked by inhaled sulphur dioxide 3) postvagotomy: when all pulmonary receptors input was removed by bilateral vagotomy.

Emphysema increased static lung compliance in rabbits (normal (N): 4.5 ± 1.5 ml/cmH₂O, $n=25$ vs. emphysematous (E): 6.4 ± 2.4 , $n=35$, $p \leq 0.05$) and rats (N: 0.24 ± 0.02 ml/cmH₂O, $n=10$ vs. E: 0.49 ± 0.06 , $n=10$, $p \leq 0.05$). It also increased mean linear intercept in rabbits (N: $79.71 \pm 8.42 \mu\text{m}$, $n=25$ vs. E: 99.62 ± 2.32 , $n=35$, $p \leq 0.05$) and rats (N: 80.5 ± 2.9 , $n=10$ vs. 108.4 ± 3.2 , $n=10$, $p \leq 0.05$).

Conscious emphysematous rabbits showed no statistically significant change in their pattern of breathing probably as a result of the large variance. However, anaesthetised emphysematous (E) rabbits had longer t_E (N: 0.49 ± 0.02 sec, $n=25$ vs. E: 0.57 ± 0.02 , $n=35$, $p \leq 0.05$) and larger V_T (N: 11.3 ± 0.19 ml vs. E: 14.7 ± 0.53 , $p \leq 0.001$) when compared to normal (N) animals. Conscious emphysematous rats showed similar changes in their pattern of breathing when the variables before and after

induction of emphysema were compared i.e. longer t_E (N: 0.31 ± 0.06 sec, $n=10$ vs. E: 0.4 ± 0.07 , $n=10$, $p \leq 0.01$) and larger V_T (N: 2.02 ± 0.3 ml, $n=10$ vs. E: 2.45 ± 0.3 , $n=10$, $p \leq 0.01$). The longer t_E was thought to be produced by more active SARs. The increase in V_T could have been a result of the larger respiratory drive in emphysematous animals.

In normal rabbits removal of SARs activity by SO_2 administration reduced ventilation, \dot{V}_I ($-18 \pm 2.2\%$) and phrenic slope, G ($-22.2 \pm 3.7\%$). However, in emphysematous rabbits, \dot{V}_I ($7.8 \pm 3.3\%$) and phrenic G ($10.8 \pm 6.6\%$) were increased. This greater drive to breathe in emphysematous rabbits is thought to be vagally mediated, for bilateral vagotomy reduced \dot{V}_I and phrenic G in emphysematous rabbits (\dot{V}_I : $-19.2 \pm 2\%$, G : $-23.3 \pm 2.1\%$), whilst it did not change those of normal rabbits (\dot{V}_I : $-3.8 \pm 2.5\%$, G : $-4.8 \pm 4.8\%$). More active rapidly adapting receptors (RARs) could be mediating this stronger drive to breathe as it was shown that the deflation reflex is stronger in emphysematous rabbits.

Inflation with $10\text{cmH}_2\text{O}$ produced a longer Hering Breuer (HB) inflation reflex (calculated as $\Delta \%$) in normal compared to emphysematous rabbits, though this was not statistically significant (N: 840.18 ± 236 , $n=25$ vs. E: 623.86 ± 92.2 , $n=35$). Increased activity from RARs or C-fibres in emphysematous rabbits would shorten the HB inflation reflex. Pre SO_2 , deflation with $10\text{cmH}_2\text{O}$ increased H of the 2nd and 5th breaths (calculated as $\Delta \%$) more in emphysematous than in normal rabbits (2nd N: 16.53 ± 4.45 vs. E: $32.25 \pm 4.$, $p \leq 0.05$; 5th N: 12.46 ± 3.42 vs. E: 29.53 ± 4.06 , $p \leq 0.05$). Post SO_2 , deflation with $10\text{cmH}_2\text{O}$ increased H of the 1st, 2nd and 5th breaths (calculated as $\Delta \%$) more in emphysematous than in normal rabbits (1st N: 13.52 ± 4.39 vs. E: 32.6 ± 4.27 , $p \leq 0.01$; 2nd N: 15.76 ± 3.59 vs. E: 25.93 ± 2.02 , $p \leq 0.05$; 5th N: 14.36 ± 3.7 vs. E: 24.74 ± 2.14 , $p \leq 0.01$). This greater response to $-10\text{cmH}_2\text{O}$ in emphysematous rabbits could be produced by more active RARs.

Breathing accelerated by 6% CO_2 inhalation in all three stages (calculated as $\Delta \%$) showed that emphysematous rabbits have a stronger respiratory drive indicating the relative importance of vagal and extravagal inputs (pre SO_2 , G (N): 30.9 ± 3 vs. E: 37.6 ± 1.8 , $p \leq 0.05$; post SO_2 , G (N): 24.4 ± 2.3 vs. E: 33.92 ± 1.5 , $p \leq 0.05$; postvagotomy, G (N): 27.95 ± 3.2 vs. E: 45.61 ± 2.08 , $p \leq 0.001$).

Data is consistent with an increased drive to breathe which may, in patients with pulmonary emphysema, be perceived as dyspnoea.

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ABBREVIATIONS

(\dot{V}_I) minute ventilation
 α_2 -MG: α_2 -macroglobulin
AELF: alveolar epithelial lining fluid
API: α_1 -protease inhibitors
BALF: Bronchoalveolar lavage fluid
BP: blood pressure
CC: closing capacity
cmH₂O: centimetre of water
CO: carbon monoxide
CO₂: Carbon dioxide
COPD: chronic obstructive pulmonary disease
Cst: static lung compliance
CV: closing volume
DRG: dorsal respiratory group
E: emphysematous animals
EMG: electromyography
F.S.D: full scale deflection
F: frequency
FEV₁: forced expiratory volume in one sec
FRC: functional residual capacity
FVC: forced vital capacity
H.B. : Hering-Breuer inflation reflex
Hz: Hertz (cycles per sec)
I.D.: inside diameter
l: litre(s)
Lm: mean linear intercept
min: minute(s)
ml: millilitre(s)
ms: millisecond
N: normal animals

N₂: nitrogen
NaCl: sodium chloride
NE: neutrophil elastase
O.D.: outside diameter
O₂: Oxygen
PEF: peak expiratory flow
ppm: parts per million
PPE: porcine pancreatic elastase
RAR: rapidly adapting receptor
Raw: airway resistance
RIP: respiratory inductive plethysmograph
RV: residual volume
SAR: slowly adapting pulmonary stretch receptor
sec: second (s)
SO₂: sulphur dioxide
t_E: expiration time
t_I: inspiration time
TLC: total lung capacity
t_{TOT}: time of a breath
Tl,co: lung diffusing capacity
VC: vital capacity
VRG: ventral respiratory group
V_T: tidal volume

Chapter 1

1. INTRODUCTION

1.1 THE DISEASE EMPHYSEMA

The most predominant complaint of patients with pulmonary emphysema is dyspnoea, especially on physical exertion. It was proposed that an altered respiratory drive might model the origin of the sensation of dyspnoea experienced by many patients with emphysema. Usually emphysema in human is associated with obstruction.

1.1.1 DEFINING CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is a term used to describe a form of abnormal lung function attributed to airflow obstruction in bronchi. Within the category of COPD there are two disease processes: chronic bronchitis and pulmonary emphysema. Bronchitis has been defined by the World Health Organisation as chronic or recurrent excess mucus secretion into the bronchial tree on most days for at least 3 months of the year for at least two successive years (American Thoracic Society, 1995).

COPD causes shortness of breath or dyspnoea which can result in total disability. Most patients with severe airflow limitation are restricted by breathlessness (Jones & Killian 1991). COPD is a major cause of morbidity and mortality. In the European Union, COPD and asthma, together with pneumonia, are the third most common cause of death. In North America, COPD is the fourth leading cause of death, and mortality rates and prevalence are increasing (Siafakas, Vermeire, Pride, Paoletti, Gibson, Howard, Yernault, Decramer, Higenbottam, Postma & Rees 1995). COPD is the most common chronic lung disease, affecting 14.6 million people in the U.S. of which 12.6 million have chronic bronchitis and 2 million have emphysema (American Lung Association, 1996)

Emphysema was defined by Ciba Foundation Guest Symposium (1959) as an enlargement of the acinus, that might or might not be accompanied by destruction of the respiratory tissue. Subsequently The World Health Organisation (1961) and The American Thoracic Society (1962) limited the term emphysema to enlargement of any part or all of the acinus accompanied by destruction of respiratory tissue. The

current definition of emphysema, that of The National Heart, Lung and Blood Institute Workshop (Snider, 1985) was intended to elaborate more on tissue destruction and to resolve the confusion between airspace enlargement and destruction associated with fibrosis and the process associated with clinically significant emphysema, where little fibrosis is generally observed. The workshop introduced the following definitions:

Respiratory air space enlargement was defined as an increase in airspace size as compared with the airspace of normal lung. The term applies to all varieties of airspace enlargement distal to the terminal bronchioles, whether occurring with or without fibrosis. There are three classes of airspace enlargement: 1) simple airspace enlargement, 2) emphysema and 3) airspace enlargement with fibrosis.

1) Simple airspace enlargement: in this form the pattern of the acinus is retained and there is no evidence of destruction. The process may be congenital, as in Down's Syndrome or Congenital Lobar Overinflation. It may also be acquired, as in compensatory overinflation and in the uniform respiratory airspace enlargement of the ageing lung.

2) Emphysema was defined as a condition of the lung characterised by abnormal, permanent enlargement of airspace distal to the terminal bronchioles, accompanied by the destruction of their walls, and without obvious fibrosis.

3) Airspace enlargement with fibrosis: Airspace enlargement may accompany any fibrosing lesion in the lung. The airspace enlargement occurring with interstitial lung disease such as interstitial pulmonary fibrosis is often referred to as honeycombing or end-stage lung (Colby & Carrington, 1988; Webb, Muller & Naidich, 1992).

Chronic bronchitis and emphysema differ from asthma because the airway obstruction is constant, less episodic, associated with smoking, and usually is not linked with allergic phenomena. In pure form, asthma usually occurs in the younger age groups, while COPD occurs in the older patient.

1.1.2 CLASSIFICATION OF EMPHYSEMA

Emphysema is classified anatomically into three subtypes, according to the portion of the acinus primarily involved, as observed in mild disease. An acinus is defined as the respiratory airspaces arising from a single terminal bronchi (fig. 1.1). As emphysema becomes more severe, classification into the anatomic subtypes becomes difficult. The pathological significance of the different anatomic patterns of emphysema is not known. The three anatomic subtypes are: 1) Centriacinar emphysema, 2) Panacinar emphysema and 3) Distal acinar emphysema.

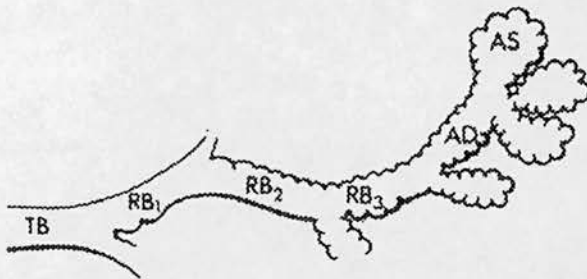


Fig. 1.1 The acinus is the gas-exchanging unit of the lung and is subtended by the terminal bronchiole (TB). The acinus consists of, in sequence, respiratory bronchioles (RB) with increasing numbers of alveoli in their walls, alveolar ducts (AD), and alveolar sac (AS).

1) Centriacinar emphysema: Centriacinar emphysema is also referred to as proximal acinar emphysema because of the proximal location of the primary part of the acinus involved, the respiratory bronchiole. Two different forms of this lesion are known. The first of these is classically associated with cigarette smoking and airflow obstruction and is known as centrilobular emphysema (CLE). CLE is so called because, when mild, its lesions appear in the centre of the secondary lung lobule; the process begins and is more severe in the upper lung zones and is more frequent in male. It is associated with parenchymal fibrosis and inflammation and distortion of the supplying bronchiole (Leopold & Gough, 1957).

2) Panacinar emphysema: Panacinar emphysema, also known as panlobular emphysema (PLE), involves the acinus in a relatively uniform manner. It is the form of emphysema associated with homozygous α -1 protease inhibitor (API) deficiency, but is commonly occurs in smokers, with normal serum proteins, in association with CLE. As CLE becomes more severe, the lesion becomes indistinguishable from PLE (Kim, Eildman. Izquierdo, Ghezzi, Saetta & Cosio, 1991). PLE also occurs in a proportion of persons in the their eighth decade and beyond. In contrast to the upper zonal predilection of CLE, PLE predominates in the lower lung zones (Snider, Brody & Doctor, 1962; Thurlbeck, 1963).

3) Distal acinar emphysema: In distal acinar emphysema the alveolar ducts and sacs are predominantly involved (Thurlbeck, 1976). Because of association of this form with secondary interlobular septa, it is also known as paraseptal emphysema (Heard, 1959). The lesion tends to occur adjacent to interlobular septa and beneath the pleura. The most common location of distal acinar emphysema is in the upper lobes, where it may be responsible for simple spontaneous emphysema in young adults (Reid, 1967; Edge, Simon & Reid, 1966).

1.1.3 EPIDEMIOLOGY OF EMPHYSEMA

Mortality rates from COPD and allied conditions vary widely between countries. Whilst the differences may be due to differences in exposure to risk factors, to a large extent methodological problems with death certification or coding account for the high country variation (Siafakas *et al.*, 1995).

There is a general consensus from investigators whose studies are centred upon the population of industrial cities, about the following: in the usual population, based on studies at autopsy, the average age of presentation is approximately 60 years with a predominance of males as two third of males were found to have emphysema and only about one fourth of females.

Emphysema is quite uncommon in people under the age of 40, rising in frequency till the seventh decade, and then declining. The average severity of emphysema is greater in males than females and there is a rapid increase in average severity from the fifth to the seventh decade and then a decline (Thurlbeck, 1991).

According to British data for 1982-1983, respiratory diseases rank as the third most common cause of days of certified incapacity; COPD accounted for 56% of days in males and 24% in females (LAIA: Lung Asthma Information Agency, 1992).

In a factsheet published by Lung Asthma Information Agency (1996) the following was reported: In men aged 65+, for the three years 1990-92, there were 51,500 deaths attributed to COPD in Great Britain, a crude mortality rate of 48.2 per 10,000. The number of lives claimed by chronic lung disease has increased sharply; in 1979 it accounted for about 50,000 deaths and in 1982, the number rose to 59,000 and by 1987, the number of deaths had reached 78,000.

1.1.3.1 RISK FACTORS

Age and Sex: Prevalence, incidence, and mortality rates for COPD increase with age, are higher in males than females, and are higher in whites than in nonwhites. Incidence and mortality are generally higher in blue-collar workers than in white-collar workers and in those with fewer years of normal education (Snider, 1989).

Tobacco Smoking: Was introduced into Europe and Britain soon after the arrival in America of Columbus in 1492. More than 2000 potentially noxious constituents have been identified in tobacco smoke, some in the gaseous phase others in particulate phase (Nunn, 1987). Smoking is so addictive that most smokers can not give up the habit (Snider, 1992). Cigarette smoking is the leading cause of pulmonary illness in the U.S. In 1990, smoking caused 84,475 deaths from pulmonary disease, mainly due to such problems as pneumonia, influenza, bronchitis, emphysema, and chronic airway obstruction (Bartecchi, MacKenzie & Schrier, 1994).

Occupational Exposure: Death rates for chronic respiratory diseases are higher than expected among men in certain occupation and industries.

Smoking effects usually confound interpretation of the occupational effects (U.S. Dept. Health and Human Service, 1984; Buist, 1988). There is controversy as to whether focal emphysema of coal workers is due to the dust accumulated within the lungs or whether the dust has accumulated in emphysematous lesions caused by other factors such as smoking.

Air Pollution: It is established that high levels of environmental air pollution are harmful to persons with chronic heart or lung disease. Although the exact role of air pollution in producing COPD is unclear, its role is small compared to that of cigarette smoking (U.S. Dept. Health and Human Service, 1984).

Miscellaneous: There is some evidence that the atopic state may predispose smokers to the development of airways obstruction. Severe respiratory infection in childhood has been invoked as a cause of diminished lung function and increased susceptibility to the effect of cigarette smoking (Higgins, 1986; U.S. Dept. Health and Human Service, 1984; Buist, 1988). Aggregation of COPD has been noted in some families without API deficiency. The genetic basis of this aggregation is not known (Buist, 1988), however, recently Sandford, Weir & Paré (1997) reviewed evidence for genetic component to COPD and have described the genes that could contribute to the genetic risk. Other risk factors which may be associated with COPD include leanness, nutrition, alcohol consumption, and climate.

Genetic Factors: The discovery by Eriksson (1964) of a family with early onset of emphysema and deficiency of α_1 -antitrypsin (α_1 -protease inhibitor) in their serum has stimulated a great deal of work in this field. It is estimated that 70,000 to 100,000 Americans living today were born with a deficiency of α_1 -antitrypsin and it has been calculated (Cook, 1971) that about 1 in 5000 children in Britain are born with the homozygous deficiency (type ZZ). In U.S. this factor accounts for only about 1% of the COPD. α_1 -protease inhibitor, a serum protein of 52 kilodaltons, is normally found in the lungs and is capable of inhibiting several types of serine proteases, including neutrophil elastase. Using electrophoretic mobility, some 30 phenotypes have been recognized in the pi system for α_1 -protease inhibitor. Homozygous (piZZ) α_1 -protease inhibitor deficiency is accompanied by premature development of severe emphysema. Heterozygotes for piZ and other variant alleles do not seem to be at increased risk of developing COPD (Buist, 1988).

1.1.4 PATHOGENESIS OF EMPHYSEMA

1.1.4.1 ELASTASE-ANTI-ELASTASE THEORY

Before 1963, emphysema was thought to be due to two factors: mechanical, and ischemic or atrophic. Mechanical factors implicated were excessive inspiratory force causing damage by stretching of lung tissue, and expiratory obstruction of bronchioles by secretion or mucosal thickening followed by dilatation of alveoli and rupture of airspace walls. The latter mechanism was thought to be the dominant one (Laennec, 1835, cited by Snider, 1992). Thrombosis and obliteration of vessels by increased alveolar pressure were invoked as possible causes of capillary obliteration (Waters, 1862, Cited by Snider, 1992).

In 1963, Laurell and Eriksson (1963) made an observation associating deficiency of alpha-globulin in five subjects with varying clinical presentations. Three had obstructive airway disease while two did not. The deficient serum protein was called α_1 -antitrypsin and the condition was termed α_1 -antitrypsin deficiency. But, because of the protein's broad antiproteolytic properties, it was referred to as α_1 -protease inhibitor (API). These observations strongly supported the hypothesis that genetic factors are important in emphysema pathogenesis. The true biological significance of API deficiency in emphysema pathogenesis became more evident when Gross and colleagues (1965) described emphysema development in rats after trachea instillation of papain, a proteolytic enzyme. Based on this discovery, the "protease-antiprotease theory of emphysema" came into being. This theory was later replaced with "elastase-anti-elastase theory" after it was recognized that experimental emphysema produced by tracheal instillation of protease occurred only if the proteases had elastolytic activity (Johanson & Pierce, 1972; Snider, Hays, Franzblau, Kagan, Stone & Korthy, 1974). The elastase-anti-elastase theory postulates that elastin fibres in the alveolar septa are constantly exposed to endogenous elastases, but are protected from these proteolytic enzymes by antielastases in the interstitial fluid bathing the elastin fibres. Antielastase deficiency or elastase excess disrupts the balance and destroys elastin (Rohatgi & Kuzmowych, 1993).

A complex system of antielastases works to counteract the potential damage by endogenous elastase to lung connective tissue, especially elastin. This system is divided into 3 components: 1) circulating or serum antielastases, 2) antielastases

made in the lung in general and 3) antielastases made by specific elastase-producing cells in the lung (Rohatgi & Kuzmowych, 1993).

1.1.4.2 PATHOGENESIS OF EMPHYSEMA IN α 1-PROTEASE INHIBITOR (API) DEFICIENCY

α 1-protease inhibitor (API) is coded by a 12.2 gene located on chromosome 14 at q 31-32.3 (Rohatgi & Kuzmowych, 1993). The alleles are codominant, meaning maternal and paternal genes are fully and simultaneously expressed. This gene is primarily expressed in hepatocytes, where it directs synthesis and secretion of API into the plasma.

1.1.4.3 PATHOGENESIS OF EMPHYSEMA IN CIGARETTE SMOKERS

The epidemiologic association between cigarette smoking and emphysematous destruction of the lung is strong (Janoff, 1983). According to the elastase-antielastase hypothesis, smoking affects alveolar elastin destruction by increasing the lung's elastase burden and decreasing the protective effect of antielastase in the lung (Janoff, 1983).

In summary, cigarette smoking damages lung elastin by increasing the elastase burden and creating a functional antielastase deficiency in the lung, and perpetuates the damage by inhibiting repair and resynthesis of mature elastine (Rohatgi & Kuzmowych, 1993).

1.1.5 QUANTIFICATION OF EMPHYSEMA

1.1.5.1 CLINICAL AND LABORATORY ASSESSMENT OF COPD

The pattern of breathing in emphysema can be changed by two mechanisms. a) Alteration in lung structure leading to changes in pulmonary receptor activity, which in turn may lead to changes in lung reflexes and pattern of breathing. b) By the mere sensation of dyspnoea, as it is known that many patients adapt to a pattern of breathing to minimise the sensation of dyspnoea (Pearson & Calverley, 1995; Calverley, 1995).

(i) Clinical manifestation

Patients with COPD usually come to a physician seeking relief from shortness of breath or cough. Typically, the patient is in the sixth or seventh decade of life and gives a history of cigarette smoking. The patient complains of the insidious onset of shortness of breath with exertion. Other manifestations that occur late in the course of the disease usually represent complications of COPD. For example, leg swelling suggests right-sided congestive heart failure and pulmonary hypertension.

The classic descriptions of the patient with pure chronic bronchitis (the “blue bloated”) or pure emphysema (the “pink puffer”) summarise many of the clinical manifestations of severe COPD (Panettieri, 1995). The pink puffer (type A COPD) is tachypneic. Respirations are laboured and pursed-lip breathing (tensing of the upper and lower lips which are maintained in a rounded position during expiration) is seen. Arterial oxygenation is well preserved, however. The pink puffer suffers primarily from emphysema. The blue bloater (type B COPD) is cyanotic secondary to hypoxemia. In addition, hypercapnia, leg swelling, and right-sided congestive heart failure are present. The blue bloater suffers primarily from chronic bronchitis.

Physical examination of symptomatic patients will often reveal a prolonged expiratory phase, the pursed-lip breathing and auscultation of the chest often reveals reduced breath sounds and expiratory wheezing. Use of the accessory muscles, principally the sternomastoids, at rest suggests advanced disease and/or a clinical exacerbation. Patients with advanced COPD develop progressive hyperinflation with an increased anteroposterior chest diameter; as a consequence the ribs become more horizontal and since the tracheal position is fixed by the mediastinum, there appears to be a shortening of the trachea. Moreover the trachea appears to descend with each inspiration. Cardiovascular examination may reveal the presence of pitting oedema, cyanosis and elevated jugular venous pressure (Pearson & Calverley, 1995).

(ii) Laboratory assessment

(a) Pulmonary function tests

In human beings pattern of breathing can be assessed by direct spirometry. The Vitalograph can be used to measure forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and the FEV1/FVC ratio or the body plethysmography (Kelley, 1995).

In experimental animals, the pattern of breathing is commonly recorded by measuring tracheal airflow using a pneumotachograph.

All the above methods for measuring pattern and lung volume require the subject to be connected to the instruments through a face mask or mouth piece and a nose clip, which have been shown to alter the pattern of breathing by increasing tidal volume (Gilbert, A, Baule, Peppi & Long, 1972; Askanazi, Silverberg, Foster, Hyman, Milic-Emili & Kinney, 1980; Tabachnik, Muller, Toye & Levison, 1981; Hirsch & Bishop, 1982) with an unchanged (Askanazi *et al.*, 1980; Tabachnik *et al.*, 1981) or reduced breathing frequency (Gilbert *et al.*, 1972; Hirsch & Bishop, 1982).

For these reasons and the convenience of use, alternative techniques that measure ventilation without a face mask or mouth piece and nose clip have been developed. These techniques are: 1) magnetometry, 2) canopy spirometry, 3) respiratory inductive plethysmography and 4) barometric method.

1) Magnetometry:

In this method the linear motion of the chest wall and abdomen is measured by magnetic coils (Gilbert, Auchincloss, Baule, Peppi & Long, 1971; Konno & Mead, 1967).

2) Canopy Spirometry:

This technique involves the use of a 40-liter head canopy connected to a spirometer (Spencer, Zikria, Kinney, Broell, Michailoff & Lee, 1972; Askanazi *et al.*, 1980).

3) Respiratory Inductive Plethysmograph (RIP):

The apparatus consists of insulated coils of wire encircling the rib cage (RC) and abdomen (ABD) (Cohn, Rao, Broudy, Birch, Watson, Atkins, Davis, Stott &

Sackner, 1982; Chadha, Watson, Birch, Jenouri, Schneider, Cohn & Sackner, 1982; Tobin, Chadha, Jenouri, Birch, Gazeroglu & Sackner 1983a&b).

4) Barometric Method:

This is the only method which determines tidal volume by measurement of parameters directly related to the volume of ventilation and which does not require any tactile contact with the subject. As this apparatus is most suitable for the present study, a detailed description of the method is warranted.

Chapin (1951) was the first to use the barometric method to measure the pattern of breathing in laboratory animals after observing that in a closed chamber containing a man, the pressure increases in inspiration. The thorax expands more than the volume of the gas inspired from the chamber because inspired air is heated and water vapour is added to it; this increase in pressure will be transmitted to all parts of the chamber. A decrease in pressure during expiration is due to the cooling and drying of the expired air in the chamber. The method was sufficiently sensitive to record tidal volume in unanaesthetised hamsters in the range of 1-3ml at frequencies of 20-120/min. The method has therefore been utilised in investigating the effects of autonomic drug on ventilation of small animals (Tawab, 1950).

Drorbaugh & Fenn (1955) elaborated the method with the intention of using it to measure the pattern of breathing in premature infants. The simultaneous recording of breathing by barometric and pneumotachograph methods demonstrated that changes in flow at the onset of inspiration and at the end of expiration are recorded by the barometric method within 0.1 sec.

The method was further modified by Bartlett & Tenney (1970) by adding a reference chamber which was connected to the animal chamber with a slow leak which made the system independent of minor pressure changes in the laboratory. The method was then used to investigate the effects of anaemia on ventilation in rats and cats.

Epstein & Epstein (1978) found small discrepancies in the apparent times of onset and end of inspiration; in general pneumotachography indicated that inspiration started approximately 50ms earlier and 50ms later than indicated by the barometric method.

Lucey *et al.* (1982) utilised the barometric method to study the effect of emphysema on the pattern of breathing of hamsters. They felt justified to use the original formula of Drorbaugh & Fenn (1955) without the correction suggested by Epstein & Epstein (1978) because applying the same correction to control and treated animals would not influence the comparison between the two groups.

An animal's pattern of breathing (t_i , t_E , V_T) and FRC can be measured by adapting the above methods.

(b) Airway Resistance and Pulmonary Compliance

Airway resistance (R_{aw}) is related to the pressure difference between the alveoli and the mouth per unit of airflow. It can be accurately determined using the body plethysmograph (Kelley, 1995).

In experimental animals, R_{aw} is commonly measured during normal breathing. In this method lung volume, flow rate and intrapleural pressure are measured simultaneously (West, 1985). The resistance calculated in this way is called pulmonary resistance since it comprises airway resistance plus tissue resistance. Because intrapleural pressure reflects two sets of forces, the component that opposes the elastic recoil of the lung should be subtracted to leave the pressure that was used to overcome pulmonary resistance. Subtraction can be performed mathematically (Gallivan & McDonell, 1988) or electrically (Nadel & Widdicombe, 1962; Davies, Dixon, Callanan, Huszczuk, Widdicombe & Wise, 1978).

Compliance ($\Delta V/\Delta P$) is defined as the volume change per unit of pressure change across the lung. It is either measured during no-flow conditions where the changes in transpulmonary pressure (abscissa) is plotted against changes in lung volume (ordinate) and the slope of the linear portion of the graph is taken as static compliance (C_{stat}) or it can be measured under conditions of air flow and the slope of the line drawn between the end-inspiratory and end-expiratory points of the dynamic pressure-volume curve is taken as the dynamic compliance (C_{dyn}) (Grippi, 1995a). In the present study the static method was adapted to measure lung compliance. With normal airway resistance, C_{dyn} approximates C_{stat} and does not vary significantly with respiratory rate.

(c) Inequality of Ventilation and Lung Diffusing Capacity

Inequality of ventilation can be assessed by a single breath N_2 test (Grippi, 1995b). The subject takes a single maximal inspiration of O_2 and then exhales slowly to residual volume. N_2 is measured by a nitrogen meter connected to the mouth piece and four phases can be recognised. It has been found that phase III of N_2 curve to be changed in emphysema.

The multibreath N_2 washout test can also be used to assess inequality of ventilation (West, 1987). The subject is connected to a source of pure O_2 and a N_2 meter records the end-expiratory value of nitrogen concentration in each breath. For a normal lung a straight line is obtained when $\log N_2$ concentration is plotted against breath number.

Lung diffusing capacity (Tl_{co}) is also affected by emphysema and can be assessed by using carbon monoxide (CO), because transfer of CO is solely diffusion limited (Grippi, 1995c). There are three CO methods for measuring Tl_{co} : 1) steady-state method, 2) single-breath method, 3) rebreathing method.

Lung function changes in emphysema

Humans

In emphysema, lung volumes show hyperinflation, with an increase in the ratio of residual volume/total lung capacity (RV/TLC). TLC is normal or raised and some investigators have (Osborne, Hogg & Wright, 1988; West, Nagai & Hodgkin, 1987) whilst others have not (Bergin, Muller & Miller, 1986; Morrison, Abboud & Ramadan, 1989) demonstrated significant correlation in measurement of TLC with the pathological scores of emphysema. Static lung compliance is increased in emphysema while it is normal in chronic bronchitis.

Air flow obstruction is revealed as low forced expiratory volume in 1 sec/ forced vital capacity (FEV_1/FVC) ratio and low peak expiratory flow (PEF) and it is severe in emphysema whilst it is moderate in chronic bronchitis (Panettieri, 1995).

The Tl_{co} is often well preserved in patients despite severe airway obstruction and overinflation (Dubois, 1962; Shepard, Cohn, Cohen, Armstrong, Carroll, Donoso & Riley 1955), though it may be reduced.

Tobin *et al.* (1983b) using respiratory inductive plethysmograph found elevated f , V_T , \dot{V}_{min} in both eucapnic and hypercapnic groups compared to normal, t_i was shortened and t_i/t_{TOT} was reduced. However, Loveridge, West, Anthonisen & Kryger (1984) using the same method while subjects are seated found that COPD patient had higher \dot{V}_{min} and f , and t_i was decreased but V_T and t_i/t_{TOT} were not changed.

Animals

Induction of emphysema with either papain or pancreatic elastase in a variety of animal species generally produced increases in static lung compliance, functional residual capacity (FRC), residual volume (RV) and TLC and airflow limitation (Snider, Lucey & Stone, 1986). Hachenberg, Wendt, Schreckenber, Meyer, Hermeyer, Muller & Lawin (1989) found that papain-induced emphysema in rats increased closing volume (CV), vital capacity (VC), closing capacity (CC) and the phase III of N_2 washout curve was steeper compared to normal. Likens & Mauderly (1982) found similar increases in CC and VC, but phase III of the N_2 washout curve was not changed. Airway resistance was not altered in rats (Boyd, Fisher & Jaeger 1980) and dogs (Marco, Meranze, Yoshida & Kimbel 1972; Hachenberg *et al.*, 1989) treated with papain suggesting that the central airways were unaffected. Tl_{CO} was found to decrease progressively as the dose of papain increased (Pushpakom *et al.*, 1970; Damon, Mauderly & Jones 1982). Marco *et al.* (1972) showed that papain-induced emphysema in dogs increased V_T and decreased \dot{V}_{min} slightly. Damon *et al.* (1982) showed that elastase-induced emphysema in rats increased V_T and \dot{V}_{min} while Delpierre, Fornaris & Payan (1985) showed that in rabbits it decreased V_T and increased t_i . The conscious pattern of breathing of emphysematous animals was compared to normal animals in two species. Gillespie, Tyler & Eberly (1966) found that emphysematous horses had lower t_i and V_T , and higher R_{aw} compared to normal horses. Lucey, Snider & Javaheri (1982) using the barometric method, found that hamsters treated with elastase for one month had a similar pattern to untreated animals, whilst 5 & 13-months elastase treated had higher V_T , t_i and t_E compared to untreated animals.

1.1.5.2 PATHOLOGICAL DIAGNOSIS

(i) Fixation

The aim of fixation is the preservation of cells and tissue constituents in a condition identical to that existing during life and to do this in a way that will allow the preparation of thin, stained sections. In practice, the purpose of fixation is: (1) to prevent or arrest autolysis and bacterial decomposition and putrefication; (2) to so coagulate the tissues as to prevent loss of easily diffusible substances; (3) to fortify the tissue against the deleterious effects of the various stages in the preparation of sections, e.g. dehydration, clearing, and wax impregnation (tissue processing); and (4) to leave the tissues in a condition which facilitates staining with dyes and other reagents.

The three principle methods of lung fixation are 1) the negative pressure formalin steam method of Weibel & Vidone (1961), 2) the constant - pressure airway instillation of fixative described by Heard (1958) (a modification of which is used in this study), 3) the technique recommended by the American Thoracic Society (ATS) (Krahl, Tobin, Wyatt & Loosli, 1959) which involves inflating the lungs by airway instillation until their 'natural contours' are established and then floating them in fixative.

The formalin steam method is technically demanding and was criticised by Wright, Slavin, Kreel, Callan & Sandin (1974). It was found that in the method recommended by the ATS that fixed lungs will flatten and shrink as fixative leaks through the pulmonary vessels.

Most body tissues shrink during both fixation and histological processing and a constant can be calculated to correct for this artifact if interstudy comparisons are of a primary concern. Where all material is treated in an identical fashion, as in this study, such corrections are largely irrelevant (Aherne & Dunnill, 1982).

(ii) Sampling of the lung

In any histological analysis of the lung, sampling is all important. It is an important premise that the selection of these samples must ensure that they give a picture of the whole organ, not just of the "interesting areas".

Two methods are available. The first is systematic sampling, and this involves taking blocks of tissue at given intervals throughout the lung. It's main disadvantage is that, if the diseased areas have a similar type of arrangement to the sampling pattern, the sample will be unrepresentative of the lung as a whole, and any results deduced from such samples will be hopelessly wide of the true state of affairs. The alternative is random sampling, the selection of blocks by means of a random number table. In theory this method has the property of securing a small group of blocks of tissue possessing the same characteristics as the entire lung, i.e., the same proportion in which each special feature is present or absent. In practice in a totally random sample, where each of the sample units has an equal chance of selection, a highly unrepresentative selection is possible, e.g., two adjacent blocks of tissue from the upper lobe and no blocks from the lower lobe in a given slice of lung. To prevent the occurrence of this type of selection a compromise between random and systematic sampling is employed. This is the principle of stratified random sampling, by which random samples are taken systematically from sections distributed throughout the lung. This retains the advantage of random sampling but imposes some restrictions on the fluctuations encountered in a simple random sample (Dunnill, 1962). These methods are particularly useful for sampling large lungs such as those of the humans where it is often not practical to examine the entire lung. In lungs from small animals it is often feasible to examine the whole, or a large portion of the lung reducing the need for sampling.

(iii) Assessment of emphysema

Emphysema was defined as a condition of the lung characterised by abnormal permanent enlargement of airspaces distal to the terminal bronchiole, accompanied by the destruction of their walls. One of the widely used measurements to assess air space enlargement is that of mean linear intercept (L_m) which is the average distance between alveolar walls. Dunnill (1962) used L_m to calculate internal surface area of the lung. Tomkeieff (1945) who was interested in the grain size of rocks, appears to have been the first to have drawn attention to the method of finding the surface area of small objects from their areas of projection. This method was developed by

Campbell & Tomkeieff (1952) to include a number of small bodies such as alveoli in the lung.

In the application of this method to the lung Dunnill (1962) stressed that the traverses best employed for this work are crossed hair lines, of equal and known length fitted to an eyepiece. The use of the two hair lines, at right angles to each other would compensate for any deformation that may occur to the section during cutting and mounting. The number of intercepts are counted on both the horizontal and the vertical lines for each field. A cut through an alveolar wall counts as a single intercept, while a cut into a blood vessel wall counts as half, as does a cut out of a blood vessel wall. The mean linear intercept L_m is then calculated from m , the sum of all the intercepts, L , the length of the traverses, and N , the number of times the traverses are placed on the lung, *i.e.* in this case twice for each field examined $L_m = N.L./m$. This was the method adopted for the present investigation.

In the diagnosis of pulmonary emphysema, Thurlbeck (1967) compared some subjective measurements: a subjective visual assessment (units), an assessment of the volume of the lung parenchyma (point count), an average subjective visual grading by eight pathologists (Co-op score), to the objective measurements, namely the mean linear intercept (L_m), L_m corrected to total lung capacity (L_{mc}), internal surface area at 25 cmH₂O. transpulmonary pressure (ISA), and ISA at total lung capacity (ISA_s). Thurlbeck (1967) concluded that objective measurements parallel the subjective measurements of emphysema and correcting L_m and ISA to the measured total lung capacity did not particularly improve the relationship over that of L_m and ISA and that L_m may have an advantage over ISA in recognising mild grades of emphysema.

To assess the degree of alveolar wall destruction in emphysema, Saetta, Shiner, Angus, Kim, Wang, King, Ghezzi & Cosio (1985) used the destructive index (DI) measurement, which measures the percentage of the ducts and alveoli that have been destroyed as follows: $DI = D / (D+N) \times 100$, where D is the destroyed and N is the normal lung. In comparing DI to L_m in elastase-induced emphysema, Eidelman, Bellofiore, Chiche, Cosio & Martin (1990) concluded that DI was merely a subjective measure of airspace enlargement that provides essentially the same

information as Lm and this reflects the predominance of air space enlargement over alveolar septal damage as the underlying morphologic abnormality in this model.

1.1.5.3 RADIOLOGICAL DIAGNOSIS

(i) Plain chest radiograph

Plain chest radiography has been suggested as a diagnostic tool in emphysema. However the extent of emphysema diagnosed radiographically is poorly correlated with the severity of lung changes found at necropsy (Thurlbeck & Simon, 1978). At the presentation of a patient with COPD, the plain chest radiograph can exclude other conditions such as carcinoma of the bronchus and it may also suggest cor pulmonale and pulmonary hypertension. In acute exacerbation of COPD, a chest radiograph is important to confirm or exclude complicating pneumonia or a pneumothorax.

(ii) Computed tomography (CT)

Computed tomograph provides a means of measuring tissue density. Emphysema reduces the lung density, and this can be visualised as low attenuation areas on the CT scan. The finding can be quantified by measuring the frequency distribution of density values from each picture element (Gould, MacNee & McLean, 1988). Recent evidence suggests that high resolution CT scanning is sufficiently sensitive to diagnose emphysema in patients with normal chest radiographs and an isolated low diffusing capacity (Klein, Gamsu, Webb, Goden & Muller, 1992). It appears to be useful in identifying patterns of emphysema, such as centriacinar and panacinar emphysema (Guest & Hansel, 1992).

Radiological diagnosis is a very important diagnostic tool in the clinical situation where pathological diagnosis is limited to the postmortem examination. However, in experimental animals, as in the present study, the diagnosis of emphysema can be acceptably performed in postmortem samples i.e. through pathological diagnosis.

1.2. ANIMAL MODELS OF PULMONARY EMPHYSEMA

1.2.1 CRITERIA REQUIRED FOR AN ANIMAL MODEL OF PULMONARY EMPHYSEMA

Because the reliable production of a reproducible model of emphysema is a necessary requirement for this study, it is worthwhile presenting a comprehensive review of the methods which have been used to produce the disease. Because emphysema is defined using anatomical terms, anatomical criteria must have the highest diagnostic priority. The evaluation of respiratory air-space size requires that the lungs be fixed in inflation, preferably at a known and standard transpulmonary pressure. Subsequent to this procedure histological sections 5-7 μ m thick, made after paraffin embedding, can be prepared and examined to detect any emphysematous changes which have been produced. An estimation of air-space size may be made in such sections by measurement of the mean linear intercept (Karlinsky & Snider, 1978), which is the average distance between alveolar walls.

Physiological measurements provide necessary supportive evidence for a diagnosis of emphysema. Increased static lung compliance and increased lung volume at a standard transpulmonary pressure (25cmH₂O) can be readily measured in anaesthetised animals or in excised lungs and provide useful, additional, diagnostic criteria (Karlinsky & Snider, 1978).

1.2.2 TECHNIQUES FOR THE PRODUCTION OF ANIMAL MODELS OF EMPHYSEMA

There is no ideal animal model of pulmonary emphysema, however, the usefulness of an experimental model should be judged on how well it answers the specific questions which are asked in particular investigation (Snider *et al.*, 1986).

Efforts to construct an experimental form of the disease date back more than a century. The crudeness of the early experimental models reflected the very limited understanding of emphysema that then existed (see reviews by Snider *et al.* 1986; Canto & Turino, 1991). Mechanical obstruction of airways was tried in the belief that that disease was primarily due to a blockage of airflow. This approach produced localised distension of air spaces but not emphysema. The development of more representative models of the disease awaited the recognition that enzyme-induced

damage to pulmonary connective tissue components, particularly elastic fibres, was of critical importance in the destruction of alveolar walls (see review by Canto & Turino, 1991). Papain and later, elastase were used to produce lesions that more closely approximated the morphological and physiological features of emphysema (see review by Canto & Turino, 1991).

1.2.3 EMPHYSEMA PRODUCED BY EXOGENOUS PROTEASES

In the exogenous protease methods, the proteases were administered by their instillation into the trachea of the animal concerned.

1.2.3.1 PAPAIN INDUCED EMPHYSEMA

The use of papain was originally intended as a possible treatment for the condition of pulmonary fibrosis (Gross, Babyak, Tolker & Kaschak 1964). However, it was found that morphologically, papain induced lung lesions resembled human panacinar emphysema. Air space enlargement was accompanied by increases in total lung capacity, functional residual capacity, and compliance (Giles, Finkel & Leeds, 1970; Pushpakom, Hogg & Woolcock, 1970). In addition there was also damage to elastic fibres, which remained disrupted despite their resynthesis (Goldring, Greenburg & Ranter, 1968; Osman, Keller, Cerreta, Leuenberger, Mandl & Turino, 1980). Consequently, lung recoil is not restored back to pre-treatment levels.

1.2.3.2 ELASTASE INDUCED EMPHYSEMA

Introduction of either pancreatic or leukocyte elastase into the lung via intratracheal instillation causes the rapid destruction of pulmonary elastic fibres, resulting in diffuse air space enlargement (Janoff, Sloan, Weinbaum, et al., 1977; Senior, Tegner, Kuhn, Ohlsson, Starcher & Pierce, 1977; Kaplan, Kuhn & Pierce, 1973). The lesion caused by neutrophil elastase is less severe than that produced by pancreatic elastase. A large increase in the urinary excretion of desmosine crosslinks (a metabolic breakdown product of elastin) during the first 24 hours following instillation of pancreatic elastase reflects biochemical degradation of elastic fibres (Kuhn, Engleman, Chraplyvy & Starcher 1983). Marked resynthesis of the elastic fibres occurs almost immediately (Osman, Kaldany & Cantor *et al.*, 1985) and, by 3

weeks, their content in the lung has returned to normal (Kuhn & Starcher, 1980). However gross tissue damage is irreversible, and may progress for months, despite the fact that nearly all of the instilled elastase is quickly cleared from the extracellular compartment (Snider & Sherter, 1977; Morris, Stone, Snider *et al.*, 1983). The physiological alterations associated with such a model are increases in total lung capacity, functional residual capacity, and static compliance along with a reduction in arterial oxygen tension (Snider & Sherter, 1977; Pereiras, O'Brien & Snider, 1977). The simplicity of production of this model and its morphological and physiological resemblance to human panacinar emphysema has resulted in its widespread use in the study of pulmonary emphysema (Canto & Turino, 1991).

1.2.4 EMPHYSEMA PRODUCED BY ENDOGENOUS PROTEASES

In the endogenous protease technique, the intratracheal administration of chemicals results in the activation of neutrophils in the pulmonary interstitium and the release of lysosomal enzymes (including elastase) by the cells

1.2.4.1 CADMIUM CHLORIDE/BETA-AMINOPROPIONITRILE-INDUCED EMPHYSEMA

Intratracheal instillation of cadmium chloride induces lung disease that is morphologically similar to interstitial pulmonary fibrosis, however the concomitant administration of Cadmium Chloride with the elastin crosslink inhibitor, beta-aminopropionitrile (BAPN), results in injury that more closely resembles emphysema (Niewoehner & Hoidal, 1982). The damage coincides with migration of large numbers of neutrophils to the lung which release lysosomal enzymes. After the first week, this reaction subsides and is replaced by a sustained influx of mononuclear cells (see review by Canto & Turino, 1991).

The inflammation produced is accompanied by diffuse enlargement of air spaces and formation of thin walled, subpleural bullae, which are prone to rupture.

1.2.4.2 NITROGEN DIOXIDE-INDUCED EMPHYSEMA

The prolonged inhalation of as little as 30 parts per million (ppm) of nitrogen dioxide causes focal air space enlargement (Kleinerman & Ip, 1979). The lesions

produced most closely resemble centriacinar emphysema. The disease is believed to result mainly from the release of elastase by neutrophils migrating into the lung; some studies suggest that elastic fibre repair may also be impaired (Blank, Glasgow, Pietra, Burdette & Weinbaum 1988).

1.2.4.3 ENDOTOXIN-INDUCED EMPHYSEMA

Administration of endotoxin initiates an acute inflammatory reaction that degrades the pulmonary extracellular matrix (Blackwood, Cantor, Moret, Mandl, Turino, 1983). Endotoxin is a neutrophil priming agent which leads to the activation of neutrophils in the pulmonary vasculature. In the presence of complement C5a, leukotrienes B₄ and interleukin 8, the neutrophils release lysosomal enzymes, oxygen radicals, prostaglandins and leukotrienes, which lead to pulmonary damage. The resultant increase in air space enlargement is relatively small and occurs only after repeated infusion of endotoxin (Guenter, Coalson & Jacques, 1981). This model has been used to demonstrate the role of alpha₁-antiprotease (A₁-PI) in preventing the pulmonary injury. If galactosamine is administered to disrupt synthesis of the inhibitor A₁-PI in the liver, the severity of endotoxin-induced emphysema is increased (Blackwood, Moret, Mandle & Turino, 1984).

1.2.5 EMPHYSEMA PRODUCED BY IMPAIRED ELASTOGENESIS

1.2.5.1 COPPER DEFICIENCY-INDUCED EMPHYSEMA

It has been found that restriction of the intake copper can lead to emphysema (O'Dell, Kilburn & McKenzie, 1978), which is believed to be due to inhibition of lysyl oxidase, the enzyme responsible for cross linking reaction in elastin and collagen. Using this technique, increases in both lung volume and static compliance are observed. Surprisingly, no significant alteration in lung elastic fibre content has been observed (Soskel, Watanabe, Hammond, Sandberg, Renzetti & Crapo, 1982), indicating that the underlying mechanism of injury in the copper deficiency model is not as simple as was once thought.

1.2.5.2 EMPHYSEMA IN THE BLOTCHY MOUSE

It has been shown that diffuse air space enlargement occurs spontaneously in the blotchy mouse as a result of a genetically induced loss of lysyl oxidase activity. A secondary abnormality in macrophage function may be involved (Fisk & Kuhn, 1976; Ranga & Kleinerman, 1981). Alveolar distension has been observed very shortly after birth which becomes progressively more severe as the animal matures. Both lung volume and static compliance are increased. Damage to the elastic fibres could be the cause of emphysematous changes observed but this needs to be confirmed biochemically (Fisk & Kuhn, 1976).

1.2.6 MODELS OF UNCERTAIN PATHOGENESIS

1.2.6.1 STARVATION-INDUCED EMPHYSEMA

Starvation of newborn animals causes generalised impairment of organ growth, including a marked reduction in lung volume and weight (Lechner, 1984; Lechner, 1985). In contrast, mature animals develop less severe lung disease characterized by an increase in alveolar size (Sahebjami & Wirman, 1981). The cause of the airspace enlargement has not been determined, although it may result from an insufficient repair response to normal degradative processes (see review by Canto & Turino, 1991). Similar changes can be produced by restricting the intake of specific nutrients, such as lysine (Kerr, 1989).

1.2.6.2 EMPHYSEMA IN THE TIGHT-SKIN MOUSE

This is another genetic model of emphysema where the underlying biochemical abnormality is unknown. Alveolar dilatation develops 6 to 8 weeks after birth and is associated with increases in both lung volume and static compliance (Szapiel, Fulmer, Hunninghake, Elson, Kawanami, Ferrans & Crystal, 1981; Kueppers, 1987).

1.2.7 PRESENT STUDY

In the present study pulmonary emphysema was produced in rats by intratracheal administration of papain, since this substance has already been successfully used to produce model of pulmonary emphysema in our laboratory

(Pirie, 1997). However when the same technique was performed in rabbits, it was found to be associated with a high mortality rates, which was thought to be due to differences in species response to papain. For this reason, pancreatic elastase was used to produce model of emphysema in rabbits. Both models are simple to produce, resulting in pulmonary emphysema which resembles human panacinar emphysema both morphologically and physiologically (Canto & Turino, 1991; Delpierre *et al.*, 1985).

Rabbits were chosen for the study of the respiratory drive in emphysema because they were extensively used in the studies of respiratory physiology, therefore a lot is known about their pulmonary receptors and reflexes, and they are the only species where SO₂ was found to block SARs selectively.

1.3. CONTROL OF BREATHING

In mammals, ventilation of the lungs results from rhythmic contractions of the diaphragm, intercostal, and abdominal muscles. The pressures generated by these “pump” muscles, combined with the flow resistance of the airways and their elastic properties, cause changes in lung volume.

Motoneurons driving the respiratory pump muscles are located at various levels of the spinal cord. 1) phrenic motoneurons innervating the diaphragm at C3-C5 (Bianchi *et al.* 1995). 2) motoneurons innervating the intercostal muscles at T1-T12 (Larnicol, Rose, Marlot & Duron, 1982; Lipski & Martin-Body, 1987). 3) motoneurons innervating the abdominal muscles at T4-L3 (Holstege, Neerven & Evertse, 1987; Miller & Wilson, 1983).

Breathing in mammals relies on a neuronal network located within the brain stem. Although the basic elements making up this network are located within the brain stem, structures outside the brain stem can and do affect respiration, but they are not essential to the network because their elimination does not impair rhythmogenesis. These elements external to the network are reminiscent of various structures previously called “centres”. The earlier belief that specific respiratory functions resided within circumscribed structures (e.g., the pneumotaxic centre) has been modified, by using the term central pattern generator (CPG) (Delcomyn, 1980) or central rhythm generator (Sears, 1990).

A special feature of the respiratory CPG is that it functions automatically but can be controlled voluntarily. In this way, respiration, as a sensory-motor act, can be modulated much like posture and locomotion and, in the context of this study, be influenced by sensation such as dyspnoea. The CNS processes afferent inputs to provide an appropriate motor output. In the respiratory system, chemosensitive, pulmonary, and even proprioceptive afferents determine the appropriate respiratory output to maintain homeostasis. Voluntary control involves the forebrain, with information being distributed to premotoneurons and motoneurons through pyramidal and extrapyramidal pathways. In contrast, automatic control involves the brain stem, the output of which is distributed to interneurons and motoneurons through proprio-brain stem pathways to cranial motoneurons, and through pathways in the ventrolateral spinal cord to interneurons and motoneurons (Aminoff & Sears, 1971). The two systems are separated by the conscious and anaesthetised parts of my study.

1.3.1 AUTOMATIC CONTROL SYSTEM

To identify the neurones responsible for the generation of respiratory rhythm and their connections, investigators (Cohen, 1979; Bianchi, Denavit-Saubié & Champagnat, 1995) have used techniques such as antidromic stimulation, microstimulation, histochemical labelling (Horseradish peroxidase or tritiated amino acids), cross correlation, and spike-triggered averaging (STA). Antidromic mapping and labelling studies provide information about possible axonal projections of respiratory neurones but do not indicate how various respiratory neurones are synaptically connected. Readers are referred to reviews (Cohen, 1979 & 1981; Bianchi *et al.*, 1995; Euler 1983 & 1986; Mitchell & Berger 1975 & 1981; Richter 1982, Cohen & Feldman, 1977; Feldman, Cohen & Wolotsky, 1976) for a detailed description.

1.3.2 VOLUNTARY CONTROL SYSTEM

Voluntary control can be cortically driven during certain normal behavioural acts such as vocalization, eating, or expression of fear or anger (Shneerson, 1988). Automatic control is temporarily interrupted due to cortical inhibition, and

coordinated respiratory activity is achieved through corticospinal and corticobulbar tract projections. Corticospinal pathways descend from motor cortex, decussate at the pyramids in the caudal medulla, and synapse on interneurons in the spinal cord that influence α -motoneurons (lower motor neurones) of the phrenic and intercostal nerves. The tract is located in the lateral columns of the spinal cord white matter. Corticobulbar fibres synapse in the brain stem motor nuclei of cranial nerves IX, X, and XI located in the nucleus ambiguus in the medulla. This allows for voluntary control over the upper airway muscles of the pharynx and larynx (Shneerson, 1988; Mitchell and Berger, 1975).

1.3.3 CONTROL OF MEDULLARY RESPIRATORY CENTRE

1.3.3.1 CHEMICAL CONTROL OF BREATHING

Because pulmonary emphysema affects the chemical composition of the blood in terms of respiratory gases, it is important to consider chemical control of breathing in any study of reflex effects of the disease on drive to breathe. The respiratory neurones in the medulla receive information on the levels of arterial P_{O_2} and P_{CO_2} in the body from the peripheral chemoreceptors i.e. the carotid and the aortic bodies (Eyzaguirre & Zapata, 1984; Pallot, 1987; Prabhakar, Mitra, Lagercrantz, et al, 1987). The carotid body cells synapse with afferent fibers of the carotid sinus nerve, a sensory branch of the glossopharyngeal nerve, the carotid sinus nerve innervates the carotid body and carotid sinus baroreceptors. Those of the aortic bodies synapse with vagal fibers. The discharge rate of the peripheral chemoreceptors increases linearly with rising P_{CO_2} but hyperbolically with hypoxia. Although the carotid body responds to changes in the arterial tension of O_2 , it is relatively unaffected by anemia or carbon monoxide. The aortic body responds more to changes in O_2 content and may have weaker response to CO_2 than the carotid body (Lahiri, Mokashi, Mulligan & Nishino, 1981).

Even in carotid body- and aortic body-denervated animals, inhaled CO_2 stimulates respiration. The site of this action continues to be debated, but evidence indicates that important sites are superficially areas near the ventrolateral medullary surface (VMS) (Bledsoe & Hornbein, 1981; Bruce & Cherniack, 1987; McAllen, 1986; Schlaefke, 1981). These studies (Bruce & Cherniack, 1987; McAllen, 1986;

Mitra *et al.*, 1986) have suggested that the main body of chemoreceptive neurons near the VMS are actually located in a superficial portion of the nucleus paragigantocellularis lateralis. Bruce & Cherniack (1987) have also shown that these neurons are not the only central chemoreceptors and there are cells elsewhere in the brain, in the DRG for example, which respond to change in CO₂.

1.3.3.2 REFLEXES EVOKED FROM THE TRACHEOBRONCHIAL TREE AND LUNGS

The major question posed in this study is whether the changes in structure seen in emphysematous lungs alter the reflex control of breathing. It is necessary therefore to review these reflexes in some detail.

There are two types of reflexes originating in the tracheobronchial tree and lungs: regulatory reflexes that determine the pattern of breathing in normal circumstances and defence reflexes that protect the respiratory tract from potentially harmful influences. Hering & Breuer (1868, cited by Widdicombe, 1961) describe the two reflexes which bear their names. They found that inflation of the lungs of dogs led to a decrease in frequency and also possibly in the force of inspiratory efforts (the inflation reflex) and that deflation of the lungs caused stronger and more frequent inspiration (the deflation reflex). Head (1889) extended this work and described a third reflex for the rabbit. With conduction in the vagus nerves blocked by a lowered temperature, inflation of the lung caused a strong sustained contraction of the diaphragm (Head's paradoxical reflex) (Widdicombe, 1961).

The importance of these reflexes in the control of breathing is not fully understood. Christie (1953) suggested the Hering-Breuer inflation reflex may adjust the rate and depth of breathing to be mechanically the most economical. Adrian (1933) concluded that the deflation reflex played no part in eupnoea in anaesthetised cat.

(i) Inflation reflex

Breuer (1868) and Hering (cited by Widdicombe, 1982) introduced the concept of the "selbststeuerung" (self regulation) of breathing via the vagus nerve. Inflation of the lungs reflexly inhibits inspiration, and deflation excites it

(Widdicombe, 1982). It is now known that the pulmonary selbststeuerung have little or no influence on the resting pattern of breathing in some species, including man. In all species, however, once a certain threshold inspiratory volume is reached, the inspiratory off-switch provided by pulmonary stretch receptor input determines V_T and t_I . Further more, in some species including dogs and rabbits, the expiratory discharge of the pulmonary stretch receptors (SARs) determines t_E and thus influences respiratory frequency at rest (Coleridge & Coleridge, 1986). The inflation reflex is abolished by bilateral vagotomy and is found to be mediated by SARs (Adrian, 1933). Vagotomy and also cooling of the vagus nerves to 8-10°C are known to slow and deepen breathing. The SARs do have a facilitatory influence early on breathing, and their inhibitory effect is latent and only appears when their discharge is sufficiently strong to switch off the phrenic discharge rather abruptly (Widdicombe, 1964). Averill, Cameron & Berger (1984) showed that SARs project to the ventrolateral nucleus of the tractus solitarius, where they make monosynaptic excitatory connections with $I\beta$ and Pump cells of the DRG. Later, Averill, Cameron & Berger (1985) showed that $I\beta$ neurones are the medullary neurones element mediating the facilitatory effect of the SARs.

(ii) Deflation reflex

Deflation of the lungs, produced either by tracheal suction, a controlled pneumothorax, or compression of the chest or abdomen, causes reflex tachypnea in dogs, cats, rabbits and guinea pigs (Coleridge & Coleridge, 1986). The vagal reflex tachypnea consists of a shortening of t_I and t_E , an increase in peak phrenic activity and also in the value of minute ventilation. The afferent route is entirely vagal, although a contribution from chest wall afferents (Bland, Lazerou, Dyck & Cherniack, 1967) and sympathetic afferents can be recognised if deflation is extreme. The reflex is blocked at a relatively high temperature (D'Angelo, Miserocchi & Agostoni, 1976; Hammouda & Wilson, 1939; Koller & Ferrer, 1970) and is thought to be mediated by myelinated afferent fibres (D'Angelo *et al.*, 1976; Davies *et al.*, 1978; Widdicombe, 1964). Results of action potential studies and anodal polarisation of the vagus nerve suggest that nonmyelinated fibres do not contribute to the deflation reflex (Guz & Trenchard, 1971). Deflation is known to reduce the SARs

activity and to increase that of RARs (Knowlton & Larrabee, 1946). The increase in breathing frequency is suggested to be due to the reduced SARs inputs (Adrian, 1933), while increase in RARs inputs is thought to lead to the increased in inspiratory drive (Coleridge & Coleridge, 1986). Davies & Kubin (1986) study showed that the ipsilateral medial and commissural subnuclei of the nucleus of the solitary tract (NTS) contained the densest arborizations and fine, presumably terminal, branches of primary afferents from RARs.

(iii) Head's paradoxical reflex

Head (1889) found that moderate lung inflation of the lungs in rabbits whose vagi were in the process of rewarming after being packed in ice, produced a sustained contraction of the diaphragm, on which rapid shallow inspiratory movements were gradually superimposed. In this instance the inflation did not lead to the usual Hering-Breuer inhibition of diaphragmatic activity which occurs when the vagus nerves are at body temperature. During this paradoxical effect, phrenic activity was found not to be augmented but was maintained at the level of a normal inspiratory effort. Some researchers ascribed a physiological significance to Head's paradoxical reflex, in causing the occasional deep breaths (Larrabee & Knowlton, 1946; Widdicombe, 1964), and possibly in reinforcing the gasps or inspiratory efforts whereby the newborn aerates their lungs (Cross, Klaus, Tooley & Weisser, 1960; Cross, 1961). Others (Coleridge & Coleridge, 1986) think that the functional significance should be confined to rabbits, because the reflex has only been shown to exist in that species. Head's paradoxical reflex is abolished by vagotomy. It is thought to be mediated by non-myelinated afferent fibres, because it can still be obtained with the vagus nerves cooled to 3-5°C (Whitteridge & Bulbring, 1944; Widdicombe, 1964; Widdicombe, 1967), a temperature that blocks transmission in myelinated fibres. It can also be obtained when transmission in myelinated fibres is blocked by anodal polarisation (Guz & Trenchard, 1971). A likely explanation of the reflex in rabbits is that pulmonary C-fibres are active during moderate lung inflation, but when the vagi are at body temperature their excitatory effects on inspiration are masked by the more powerful inhibitory input from SARs (Coleridge & Coleridge, 1986).

(iv) Gasp reflex

A large rapid inflation (Bartoli, Cross, Guz, Huszczuk & Jefferies, 1975) or large increase in airflow (Homberger, 1968, cited by Coleridge & Coleridge, 1986) in dogs was found to evoke an augmented and prolonged phrenic activity. Similar augmented breaths were evoked in cats by large rapid lung inflation (Larrabee & Knowlton, 1946). These augmented breaths are thought to be mediated by RARs (Knowlton & Larrabee, 1946). The dependence of both the irritant receptor discharge and gasp reflex on lung compliance give further support for the involvement of RARs (Reynolds, 1962). Davies & Roumy (1982) investigated the role of RAR in production of augmented breaths in response to pulses of inflation or deflation. To eliminate any role of SARs, they blocked SARs with SO₂. They concluded that RAR stimulation during inspiration produces an augmented breath followed by a refractory period for such breaths and SARs block did not suppress triggered augmented breaths; however, it made them less frequent.

1.3.4 AFFERENT NERVE SUPPLY TO TRACHEOBRONCHIAL TREE

The fibres from the tracheobronchial tree are part of the peripheral autonomic nervous system (parasympathetic and the sympathetic). Like nerve endings in the skin (Burgess & Perl, 1973; Chambers, Andres, Doring & Iggo, 1972), they consist of slowly and rapidly adapting mechanoreceptors supplied by myelinated fibres, and also of the simpler endings of nonmyelinated fibres. Although the vagus nerve is the main afferent pathway from the lower respiratory tract, afferent fibres also pass in sympathetic nerves to the spinal cord. There is evidence for afferent impulse traffic related to respiration in the sympathetic chain (Holmes & Torrance, 1976; Kostreva, Zuperku, Hess, Coon & Kampine, 1975), but no data are available on the number and size of these fibres. The role of the vagal component is generally considered most important.

1.3.4.1 SLOWLY ADAPTING PULMONARY STRETCH RECEPTOR (SARs)

Slowly adapting pulmonary stretch receptors are the afferents responsible for the Hering-Breuer inflation reflex; their mounting discharge in inspiration provides

an inspiratory off-switch, and their continuing discharge in expiration lengthens the respiratory pause (Bradley, 1977). In addition they cause tracheobronchial smooth muscle to relax and thus dilate the airways (Widdicombe & Nadel, 1963).

Slowly adapting stretch receptors appear to respond to changes in tension in the airway wall, and they display both static and dynamic properties (Bartlett, Teffery, Sant'Ambrogio & Wise, 1976; Bradley & Scheurmier, 1977; Davenport, Sant'Ambrogio & Sant'Ambrogio, 1981a). Although they adapt very slowly to maintained inflation over the long term, their discharge frequency decays appreciably within the first 1- 2sec of the abrupt onset of a stretch stimulus (Bartlett & St. John, 1979; Davenport *et al.*, 1981a; Davis, Fowler & Lambert, 1956; Knowlton & Larrabee, 1946; Widdicombe, 1954a). The initial adaptation occurs whether the lungs are inflated at constant volume (Davis *et al.*, 1956; Knowlton & Larrabee, 1946; Widdicombe, 1954a) or at constant pressure (Bartlett & St. John, 1979) and varies widely. Receptors showing pronounced adaptation can not be distinguished from rapidly receptors by their adaptation index; instead they can be identified as SARs by their remarkable regularity of interspike interval (Bystrzycka & Nail, 1980; Knowlton & Larrabee, 1946; Widdicombe, 1954a). Adaptation of a receptor is a property that is equivalent to its dynamic sensitivity and essentially depends on the viscoelasticity of the structure containing them (Davenport *et al.*, 1981a). Bartlett & St. John (1979) hypothesised that species with various breathing frequencies had different SAR adaptation rates, however their results did not support this. Recently, Davies, Pirie & Eyre-Todd (1996) proposed another mechanism for the difference in breathing frequency between species and that was the proportion of slowly adapting receptors to rapidly adapting receptors (RARs). They found that the ratio of RARs to SARs in the species sequence cat-rabbit-rat is the same as the ratio of their breathing frequency (3:4:10).

The temporal pattern of inputs from SARs influence the central respiratory mechanism in a phase-related fashion (Bradley, 1977; Trenchard, 1977). Therefore difference in the timing and pattern of receptor discharge in different regions of the airways may have functional significance. For example, SARs in the intrathoracic trachea fire phasically with each normal breath (Miserocchi *et al.*, 1973; Sant'Ambrogio & Mortola, 1977), but those in the extrathoracic trachea fire out of

phase with their intrathoracic counterparts and reach their peak frequency during expiration when transmural pressure at this site is positive. Hence extrathoracic tracheal receptors may have their main effect on expiratory time.

Few chemicals are known to excite SARs selectively. Low doses of veratrum alkaloids sensitise the receptors, and higher doses cause continuous frequency firing (Dawes, Mott & Widdicombe, 1951; Widdicombe, 1954b) probably by increasing sodium permeability (Shanes, 1958). Histamine and acetylcholine increase the response to a given stretch stimulus by contracting smooth muscle in the region of the receptor (Bartlett *et al.*, 1976; Davenport, Lee, Lee, Yu, Miller, Frazier, 1981b; Widdicombe, 1954b). The receptors appear to be more susceptible than other pulmonary afferents to inhibition by chemicals. High concentrations of volatile anaesthetics such as halothane, ether, chloroform, and trichloroethylene at first increase and then abolish receptor discharge (Coleridge, Coleridge, Luck & Norman 1968; Whitteridge & Bulbring, 1944). In rabbits (Davies, Dixon, Callanan, Huszczuk & Widdicombe, 1978) but not in cats (Grunstein, Hazucha, Sorli & Milic-Emili, 1977; Widdicombe, 1954a) high concentrations of SO₂ abolish receptor discharge selectively, an effect utilised in rabbits to assess the contribution of SARs and RARs to the deflation reflex, since both receptors are myelinated and can not be blocked differentially by vagal cooling. In addition, in preliminary experiments Davies *et al.* (1978) found that SO₂ also blocked the SARs in rats.

Inhibition of SAR activity by CO₂ is well documented, but its physiological significance is unknown. This response has been shown in rats (Schoener & Frankel, 1972) rabbits (Mustafa & Purves, 1972), dogs (Bartlett & Sant'Ambrogio, 1976; Bradley, 1976; Coleridge, Coleridge & Banzett, 1978; Sant'Ambrogio, Miserocchi & Mortala, 1974), and cats (Kunz, Kawashiro & Scheid, 1976). It is generally agreed that the inhibitory effect of CO₂ is particularly strong when administered at hypocapnic levels (Bradley *et al.*, 1976; Coleridge *et al.*, 1978; Mustafa & Purves, 1972; Sant'Ambrogio *et al.*, 1974) and the effect was shown to be less at increased transmural pressure (Bartlett & Sant'Ambrogio, 1976; Mustafa & Purves, 1972). It has been found that only bronchial SARs are susceptible to CO₂ inhalation, not those in the trachea (Bartlett & Sant'Ambrogio, 1976) and that these endings are only affected by CO₂ introduced into the bronchial lumen. The amount of inhibition

obtained by increasing airway CO₂ concentrations from levels near 0% to 7-8% was reported to be 40% of the control discharge value for receptors kept at zero transmural pressure (Bartlett & Sant'Ambrogio, 1976). The possibility that the response to CO₂ is mediated by changes in the smooth muscle tone has been extensively explored and generally denied (Coleridge *et al.*, 1978; Mustafa & Purves, 1972; Sant'Ambrogio *et al.*, 1974). The CO₂ action might be attributed to an increase in hydrogen ions, and some evidence for this hypothesis was obtained by preventing the effect of CO₂ with acetazolamide (Sant'Ambrogio *et al.*, 1974). Another hypothesis is that hypercapnic inhibition could be mediated by ionic changes in the extracellular fluid surrounding the receptors (Bartlett & Sant'Ambrogio, 1976). Release of protein bound Ca²⁺ ions during hypercapnia is another proposed factor (Fitzgerald, 1940). Coleridge *et al.* (1978) stated that hypercapnic inhibition of SARs is not specific, but rather constitutes a general characteristic of neural function.

1.3.4.2 RAPIDLY ADAPTING RECEPTORS (RARs)

After the observation by Keller & Loeser (1926, cited by Widdicombe, 1982) that the vagus nerves contained fibres from lung receptors that respond to lung inflation and deflation with a rapidly adapting discharge (unlike the slowly adapting receptors described by Adrian in 1933), Knowlton and Larrabee (1946) analysed the property of these receptors by single fibre recording. The receptors were shown to have a high volume threshold, to respond to both inflation and deflation of the lungs and by probing the airway mucosa, to have an irregular pattern of discharge and to be connected to vagal afferent myelinated nerve fibres.

Most of these receptors adapted very rapidly to maintained inflation, producing a brief irregular burst of impulses whose frequency decreased within 1sec to 20% or less of the initial discharge (Coleridge & Coleridge, 1986). These receptors were also found to be stimulated by forced deflation (Knowlton & Larrabee, 1946) and to mediate the excitatory effects evoked by large inflations and deflations. Receptors with an intermediate rate of adaptation were included in this rapidly adapting category and were distinguished from slowly adapting stretch receptors by their irregular discharge.

Widdicombe & coworkers (Mills, Sellick, & Widdicombe, 1969; Mills, Sellick, & Widdicombe, 1970; Sellick, & Widdicombe, 1971) introduced the term "irritant receptor" because of the striking response of these receptors to respiratory irritants such as ammonia, dust, and cigarette smoke. Because exogenous chemical irritants are not a physiological stimulus, the original title rapidly adapting receptors is held to be more widely applicable (Sampson, 1977; Sampson & Vidruk, 1975). The term "deflation receptors" has been used in rabbits and guinea pigs to describe receptors that are stimulated by forced deflation (Koller & Ferrer, 1970; Roumy & Leitner, 1980); these are now thought to be identical with irritant receptors (Fillenz & Widdicombe, 1972; Paintal, 1973; Widdicombe, 1981).

Rapidly adapting receptors are believed (Fillenz & Widdicombe, 1972; Widdicombe, 1981) to correspond to the epithelial nerve endings identified in the airways of several mammalian species (Elftman, 1943; Fillenz & Woods, 1970; Fisher, 1964; Larsell, 1921; Larsell, 1922). These epithelial endings are the terminal arborizations of myelinated fibres that ramify in the tracheobronchial submucosa, frequently at points of bronchial branching. The parent axon of receptors in the trachea and bronchi branches extensively to supply end formations over a wide area of mucosa (Fillenz & Woods, 1970; Fisher, 1964). The electron microscope reveals naked axons and axon enlargements between the epithelial cells of airways outside (Das, Jeffery & Widdicombe, 1978; Fillenz & Woods, 1970; Jeffery & Reid, 1973; Rhodin, 1966) and inside the lungs (Hung, Hertweck, Hardy & Loosli, 1973). These intraepithelial terminals were confirmed to be vagal afferents by degenerative experiments. Myelinated fibres have not always been identified in ultrastructural studies (Das *et al.*, 1978; Hung *et al.*, 1973), but many of the terminals are likely to represent part of a rapidly adapting receptor complex (Das *et al.*, 1978; Widdicombe, 1981).

Physiological studies in dogs (Sant'Ambrogio *et al.*, 1978) indicate that the epithelial fields of RARs may be as wide as 1cm in diameter, and that there are also sensitive terminals in the submucosal tissue since removing the mucosa does not abolish the response to deflation or inflation.

Most accounts give the general impression that RARs are inactive in eupnoea (Knowlton & Larrabee, 1946; Mills *et al.*, 1969). For example in dogs (Sampson &

Vidruk, 1975) and guinea pigs (Bergren & Sampson, 1982) with their lungs ventilated at normal resting rates and tidal volumes, average frequencies of 0.2-0.3 impulses/sec are reported. However, studies in dogs (Pack, 1981) and rabbits (Sellick & Widdicombe, 1969) indicate that their activity can be markedly increased by increasing tidal volume or airflow or both. RARs are less sensitive than SARs in providing a signal related to the degree of inflation; however they may give useful signals of airflow rates (Pack, 1981). That RARs appear to be a numerical minority does not mean they do not exert powerful effects on pattern of breathing (no one has yet demonstrated that control of breathing is a democratic process). Stimulation of a single cough receptor, which has properties similar to pulmonary RARs, profoundly disrupts breathing.

An important function of RARs may be to signal the onset of pathophysiological changes in the airways. The concept of nociceptive function was concluded from the observation that RARs in the intrapulmonary airways of rabbits are stimulated by acute pulmonary congestion, embolization, anaphylaxis, pneumothorax, and an intravenous injection of bronchoconstrictor chemicals (Mills *et al.*, 1969 & 1970; Sellick & Widdicombe, 1969; 1970 & 1971). The stimulation, produced by these experimental manoeuvres, is generally related to the increase in airflow resistance and the decrease in lung compliance (Sellick & Widdicombe, 1970), but the afferent response to a change in resistance or compliance varies widely (Mills *et al.*, 1969; Sellick & Widdicombe, 1969). The increased discharge is attributed mainly to the increased pull of a stiffened lung parenchyma on the airway walls, and this increased discharge could readily be reversed by large lung inflations that restore lung compliance (Armstrong & Luck, 1974; Sampson & Vidruk, 1975; Sellick & Widdicombe, 1970 & 1971). Deflation stimulates RARs in cats, rabbits, dogs, rats and guinea pigs and produces an irregular but well-maintained increase in firing rate (Armstrong & Luck, 1974; Knowlton & Larrabee, 1946; Koller & Ferrer, 1970; Sampson & Vidruk, 1975; Sellick & Widdicombe, 1969 & 1970).

Widdicombe (1954a) found that SO₂, a common atmospheric pollutant, sensitised approximately one-third of RARs in cats but had either little effect on or alternatively depressed the activity of the remaining receptors. High concentration of SO₂ have little effect on RARs in rabbits (Davies *et al.*, 1978) and dogs (Roberts,



Hahn, Schultz, Nadel, Coleridge & Coleridge, 1982a). The effect of SO₂ in RARs of rats is not known. Ammonia vapor has been shown to stimulate RARs in rabbits (Mills *et al.*, 1970), guinea pigs (Bergren & Sampson, 1982), and cats (Armstrong & Luck, 1974). Cigarette smoke stimulates RARs in rabbits (Sellick & Widdicombe, 1971) and guinea pigs (Bergren and Sampson, 1982). Ether stimulates RARs in guinea pigs, the effects being clearly independent of changes in lung mechanics (Bergren and Sampson, 1982). However, these three agents: ammonia vapour, cigarette smoke, ether appear to have only minor effects in dogs (Sampson & Vidruk, 1975).

Phenyldiguanide and capsaicin stimulate RARs in rabbits (Mills *et al.*, 1969) and cats (Armstrong & Luck, 1974), but the effects in cats are small and confined to less than half the receptors tested. Veratrum alkaloids stimulate RARs in much the same way as they stimulate SARs (Sant'Ambrogio & Sant'Ambrogio, 1982; Sant'Ambrogio, 1982). At least part of the increase in RAR discharge evoked by chemicals with cardiovascular effects are probably due to the stimulating effects of the exaggerated beating of the heart (Coleridge & Coleridge, 1986).

During artificial ventilation at a constant rate, RARs were challenged with an increase in CO₂ above normocapnic levels in rabbits (Sellick & Widdicombe, 1969) and dogs (Sampson & Vidruk, 1975). No significant effects were detected, even though in rabbits RAR activity tended to decrease. Coleridge *et al.* (1978) measured increases in RAR activity in dogs when airway CO₂ diminished after ligation of the pulmonary artery to the lung containing the RARs under investigation. In spontaneously breathing rabbits, an increase in inspired CO₂ activated both ventilation and RAR discharge (Sellick & Widdicombe, 1969). This increase in receptor activity was interpreted as dependent upon the inflation of the lungs to a larger volume and at a faster rate as a result of the stimulatory effect of CO₂ on respiratory output. In fact CO₂ administration to passively ventilated rabbits decreased RAR activity (Sant'Ambrogio, 1982).

If the morphological changes seen in emphysema reflexly affect the drive and pattern of breathing, it is most likely it will be through these receptor elements. Davies & Pirie (1995) have demonstrated that there is a change in pattern of

discharge in rats with papain induced emphysema. It is a major part of the present study to see if such changes produce changes in drive and pattern of breathing.

1.3.4.3 PULMONARY AND BRONCHIAL C-FIBRES

Paintal (1955) first recorded impulses in vagal afferent nonmyelinated fibres from the lungs of cats. Later Coleridge *et al.* (1965) extended these observations to dogs. Since then, action-potential studies have identified a widespread system of afferent vagal C-fibres in the lungs and airways of several species (cat, rabbit, rat), and the reflex properties of these C-fibres have been established by the use of chemicals that stimulate them selectively (Coleridge & Coleridge, 1986). Stimulation of these fibres causes rapid shallow breathing, bronchoconstriction, and increases airway secretion, often accompanied by marked cardiovascular depressor effects.

Degenerative studies in cat showed that the majority (75%) of vagal afferent fibres from the lung are nonmyelinated (Agostoni, Chinnock, Daly & Murray, 1957).

A study (Kaufman, Iwamoto, Ashton & Cassidy, 1982) that compared the responses of the various bronchopulmonary afferents to lung inflation showed that the transpulmonary pressure (cmH₂O) necessary to change activity was the lowest for SARs (5.8 ± 1.5) and highest for bronchial C-fibre endings (26.5 ± 2.9). These values were intermediate for RARs (13.5 ± 2.2) and pulmonary C-fibre receptors (16.4 ± 1.8). During spontaneous breathing, pulmonary C-fibre endings show significant activity with a respiratory rhythmicity that decreases markedly under conditions of artificial ventilation with the chest open. In contrast, the activity of bronchial C-fibre receptors is sparse and irregular during both spontaneous and artificial breathing (Coleridge & Coleridge, 1977b). Pissari, Yu, Coleridge & Coleridge (1986) studied pulmonary C-fibres and their effects on breathing in dogs. They suggested a role for receptors attached to pulmonary C-fibres in shortening expiration time in eupnoea.

In general, pulmonary C-fibre receptors show a greater response than bronchial C-fibres to mechanical events and thus are more likely to be responsible for the corresponding reflex responses. Moderate to large pulmonary inflations, introduced while nervous conduction in myelinated fibres is blocked, causes a tonic inspiratory activation with a superimposed rapid, shallow breathing (Coleridge &

Coleridge 1984; Hammouda & Wilson, 1935), bronchoconstriction (Roberts, Coleridge & Coleridge, 1982b), bradycardia, and decrease in vascular peripheral resistance (Cassidy, Eschenbacher & Johnson, 1979).

Deflation is at best a weak stimulus to afferent C-fibres from the lung and airways (Armstrong & Luck, 1974; Coleridge & Coleridge, 1977a; Coleridge, Coleridge & Luck, 1965). Neither pulmonary nor bronchial C-fibres in dogs are stimulated by deflation (Coleridge & Coleridge, 1977a; Coleridge, Coleridge & Luck, 1965). Thus afferent C-fibres differ markedly in this respect from RARs.

Dickinson & Paintal (1970) stressed the effect of CO₂ on activity in bronchopulmonary C-fibres and their possible implication in the mechanism of exercise hyperpnoea. Later, a study by Coleridge *et al.* (1978) disclosed that only a very small increase in both bronchial and pulmonary C-fibre activity occurred when CO₂ in the end tidal air of a vascularly isolated lung was raised from 19-30mmHg. Others (Delpierre, Grimaud, Jammes & Mei, 1981) found that there was a transient increase in bronchopulmonary C-fibre activity at the beginning and end of a hypercapnic stimulus. An injection into the right atrium of rabbits of sodium dithionite (a reducing agent that releases CO₂ from the blood and thus raises CO₂ concentration in the end tidal air) was found to produce a prompt and brisk activation of pulmonary C-fibres (Trenchard, Russell, Raybould, 1984). The effect of CO₂ either inhaled (Banzett, Coleridge & Coleridge, 1978; Bartoli, Cross, Guz, Jain & Noble, 1974) or added to the mixed venous blood in evoking a vagally mediated hyperpnea is well documented. The study of Russel, Raybould & Trenchard (1984) supports a role for receptors attached to C-fibres in the tachypnea that occurs during CO₂ breathing. They found that tachypnea persists after anodal block of myelinated fibres. Trenchard (1986) observed a vagally mediated increase in breathing rate due to injection of lactic and acetic acids into the right atrium of rabbits. A conclusion that pulmonary C-fibre receptors are sensitive to CO₂/H⁺ was drawn.

1.3.5 DYSPNOEA

Dyspnoea is defined as a difficult, laboured, uncomfortable breathing; it is an unpleasant type of breathing, though it is not painful in the usual sense of the word. It

is subjective and, like pain, it involves both perception of the sensation by the patient and his reaction to the sensation (Tobin, 1990).

Dyspnoea, especially on physical exertion, is the predominant complaint of patients with COPD and it is often the reason for seeking medical advice. It is probably the single most important factor that limits the ability of patients with severe COPD to function on a day-to-day basis (Eltayar, Becklake, Volta & Milic-Emili, 1996).

In normal subjects a variety of experimental methods have been employed to investigate the mechanism of dyspnoea e.g. breath holding, breathing through external resistive or elastic loads and hypercapnia.

Medullary activity and dyspnoea

It has become known that the level of central (medullary) respiratory activity or drive is an important (Altose *et al.*, 1985) perhaps the most important (Adams & Guz, 1991) component of the mechanisms that lead to the sensation of "breathlessness" through corollary discharge to the sensory cortex. In support of this mechanism is the development of air hunger during hypercapnia (1) in subjects paralysed by a neuromuscular blocking agent and ventilated via endotracheal tube (Banzett *et al.*, 1990) and (2) in tracheostomised, ventilated, high-level (C1-3) quadriplegics (Banzett *et al.*, 1989). These studies showed that feedback associated with muscular contraction, chest movement and the feedback from the upper airways are not essential for the development of dyspnoea under these conditions. These studies left only two possibilities: (1) increased medullary corollary discharge to the cortex and (2) pathways for afferent input from peripheral and central chemoreceptors that go directly to supramedullary brain and cortex, bypassing the medullary controller. Studies of children with congenital central hypoventilation syndrome where there is no increased ventilatory response to CO₂ refuted the second mechanism (Shea *et al.*, 1993). It was shown that those children did not develop dyspnoea when given CO₂ to breathe, this would have occurred if CO₂ had stimulated the cortex directly, but did when exercise led to an increase in ventilation. Therefore it seems that the medullary activity is involved and impulses from here travelling to the cortex bring about the sensation of dyspnoea.

Sense of effort and dyspnoea

The sense of muscular effort is the conscious awareness of the voluntary activation of skeletal muscle, and it arises from simultaneous activation of the sensory cortex at the time the muscles are signalled to contract (McCloskey, 1981). The sense of respiratory effort increases whenever the central motor command to the respiratory muscles must be increased e.g. when the muscle load is increased, or when the muscles are weakened by fatigue, paralysis, or an increase in lung volume. However, sense of effort can not explain dyspnoea encountered in all clinical and experimental situations, e.g. there is an increase in breathlessness if ventilation is suppressed below the level dictated by chemical drive.

Chemoreceptors and dyspnoea

Hypercapnia has long been known to cause dyspnoea. Early studies indicated such a dyspnoea arises only as a consequence of evoked changes in respiratory-muscle activity (Campbell, Godfrey, Clark, Freedman & Norman, 1969; Noble, Eisele, Trenchard & Guz, 1970). It has been found that in normal subjects who are paralysed by neuromuscular blocking agent and in patients with quadriplegia, large increases in end-tidal CO₂ produced no respiratory discomfort. However, recent work has established that hypercapnia causes dyspnoea independently of any associated reflex increase in respiratory muscle activity (Banzett, Lansing, Reid, Adams & Brown, 1989; Banzett, Lansing, Brown, Topulos, Yager, Steele, Londono, Loring, Reid, Adams, et al., 1990). It was found that ventilator-dependent patients with quadriplegia had reported air hunger when end-tidal CO₂ was raised by 7 to 11 mm Hg. Air hunger was produced by CO₂ (5 to 10 mmHg) in normal subjects who were mechanically ventilated after have been paralysed. There are several proposed mechanisms as to how hypercapnia causes dyspnoea: 1) direct projections of increased respiratory chemoreceptor activity (i.e., the carotid and aortic bodies and the medullary surface); 2) CO₂ sensitive neurones in the brain other than those which activate medullary respiratory centres; 3) a parallel copy of increased brain stem respiratory centre motor activity (corollary discharge). Results from a study by Shea, Andres, Shannon, Guz & Banzett (1993) of subjects with congenital central hypoventilation syndrome (CCHS), who therefore lack a ventilatory response to

CO₂, showed that these patients reported no respiratory discomfort during CO₂ inhalation. In addition maximal breath holding was of much longer duration than age-matched controls. These results supported mechanisms 1 & 3 and refuted mechanism 2.

It is known that normal subjects are more breathless during exercise when breathing a hypoxic gas mixture, and less breathless when breathing 100% oxygen, than they are when breathing air (Chronos, Adams & Guz, 1988). In patients with COPD, the administration of oxygen relieves breathlessness (Lane, Cockcroft, Adams & Guz, 1987), probably through a decrease in ventilation (Swinburn, Wakefield & Jones, 1984). However, there also appears to be a direct effect, independent of any change in ventilation (Lane *et al.*, 1987).

Mechanoreceptors and dyspnoea

Clinical observations suggest that upper-airway and facial receptors modify the sensation of dyspnoea. Patients sometimes notice a decrease in the intensity of their dyspnoea when sitting by a fan or open window. Studies of induced dyspnoea in normal subjects indicate that receptors in the trigeminal-nerve distribution influence the intensity of dyspnoea (Schwartzstein, Lahive, Pope, Weinberger & Weiss, 1987; Simon, Basner, Weinberger, Fencel, Weiss & Schwartzstein, 1991). The same mechanism is thought to be operative in patients with COPD in whom exercise tolerance increases and dyspnoea decreases when they breathe cold air (Spence, Graham, Ahmed, Rees, Pearson & Calverley, 1993).

The lungs contain a variety of receptors which transmit information to the central nervous system and information from these receptors may play a part in dyspnoea. Dynamic airway compression in patient with COPD may contribute to their dyspnoea, by the simple mechanical distortion of the airways during exhalation. It was found that when a negative pressure is applied at the mouth in patients with severe COPD in such a way that dynamic airway compression increases, the sensation of breathlessness increases (O'Donnell, Sani, Anthonisen & Younes, 1987). Presumably, receptors sensitive to the deformation of the airway or to changes in transmural pressure across the airway wall transmit the information that mediates the sensation of dyspnoea (Manning & Schwartzstein, 1995). Afferent information

from the lung reaches the central nervous system by way of the vagus nerve. Anaesthesia of the vagus nerve in normal subjects was found to increase breath-holding time (Noble *et al.*, 1970), and in uncontrolled studies it decreased breathlessness in some patients with cardiopulmonary disease (Guz, Nobel, Eisele & Trenchard, 1970; Davies, McQuaid, Iber, McArthur, Path, Beebe & Helseth, 1987). The role of vagal afferent activity was further elucidated by studying the sensations associated with bronchoconstriction and those associated with breathing through an external resistance. It was found that induced bronchoconstriction causes greater dyspnoea than breathing through an external resistance of comparable magnitude and inhaled lidocaine ameliorated the sensation associated with bronchoconstriction but has no effect on the discomfort associated with external resistive load (Taguchi, Kikuchi, Hida, et al., 1991). These studies suggested that information from vagal irritant receptors increases the intensity of dyspnoea and also alters its quality.

Fowler (1954) reported that the distress of breath-holding was not solely related to the chemical drive to breathe, because it was relieved by rebreathing a gas mixture containing low O₂ and high CO₂ concentrations. A role for input from pulmonary stretch receptor was proposed. Patients with heart-lung transplantation provide a unique model of chronic selective lung denervation. Some studies on these patients confirmed Fowler's results (Flume, Eldridge, Edwards & Mattison, 1996), whilst other studies refuted them (Ninane & Estenne, 1995; Harty, Mummery, Adams, Banzett, Wright, Banner, Yacoub & Guz, 1996). Masood, Reed & Thomas (1995) study refuted the claim that nebulized morphine can reduce breathlessness in COPD patients by acting locally.

Vibration of inspiratory muscles during inspiration has been shown to reduce dyspnoea associated with hypercapnia and inspiratory resistive loading in normal subjects (Manning, Basner, Ringler, Rand, Fencl, Weinberger, Weiss & Schwartzstein, 1991). Similar results were found in patients with COPD studied at rest (Sibuya, Yamada, Kanamaru, Tanaka, Suzuki, Noguchi, Altose & Homma, 1994). More recently Cristiano & Schwartzstein (1997) studied the effect of chest wall vibration on dyspnoea during hypercapnia and exercise in patients with COPD. It was found that during hypercapnia, chest wall vibration reduced breathlessness without significant changes in ventilation, however it did not significantly alter

breathlessness during exercise. These studies suggest that afferent information from the chest wall modifies the intensity of dyspnoea.

Afferent mismatch and dyspnoea

The concept of the length-tension inappropriateness as the cause of dyspnoea was proposed by Campbell & Howell (1963). According to this theory, dyspnoea arises from a disturbance in the relation between the force or tension generated by the respiratory muscles and the resulting changes in muscle length and lung volume. This theory has since been refined to incorporate the general concept of a mismatch between outgoing motor signals to the respiratory muscles and incoming afferent information (Schwartzstein, Simon, Weiss, Fencel & Weinberger, 1989; Schwartzstein, Manning, Weiss & Weinberger, 1990). A number of clinical and experimental data are consistent with this theory. In both patients and normal subjects, temporary suppression of ventilation during speaking or eating causes a mismatch between the respiratory motor command and afferent feedback from receptors in the lungs, airways, and chest wall and may cause dyspnoea. A similar phenomenon may occur in patients receiving mechanical ventilation in whom the ventilator setting selected may not match those desired by a patient with heightened respiratory drive (Manning & Schwartzstein, 1995). When normal subjects breathe an increased CO₂ concentration, their ventilation increases and most experience dyspnoea. However, if minute ventilation is reduced but end-tidal CO₂ is maintained at a constant level, the subjects report a marked increase in the intensity of breathlessness, even though the chemical drive to breathe has not changed (Chonan, Mulholland, Cherniack, Altose, 1987; Schwartzstein *et al.*, 1989). These data suggest that under a given set of conditions, the brain “expects” a certain pattern of ventilation and associated afferent feedback and that deviation from this pattern cause or intensify the sensation of dyspnoea.

Dyspnoea in COPD

When hyperinflation of the lungs occurs, the respiratory muscles become shorter and therefore less effective in generating tension. Hyperinflation may change the radius of curvature of the diaphragm, thereby placing it at a mechanical

disadvantage, which represents a load for the inspiratory muscle to overcome, as a result, respiratory motor output increases. The increased dead space ventilation means that at any given workload (and level of CO₂ production), minute ventilation will be greater in patients with COPD than in normal which necessitates an increase in respiratory motor output. The increased respiratory motor output may produce dyspnoea not only through the corollary discharge to the cortex but also through accompanying increased sense of respiratory-muscle effort (Manning & Schwartzstein, 1995). In patients with hypoxia and/or hypercapnia a chemoreceptor stimulation may well contribute to dyspnoea. Studies by Davies & Pirie, 1995; Pirie, 1997 compared pulmonary receptor activity in a rat model of emphysema to that of normal animals. It was found that SARs of emphysematous animals fired with greater peak frequency than that of normal, and RARs of emphysematous animals had greater peak frequency, greater number of spikes per second and greater number of spikes per phase during expiration. It was thus proposed that these hyperactive receptors may mediate the sense of dyspnoea experienced by many patients with emphysema.

It is the scope of the work of this thesis to investigate the drive to breathe as phrenic activity in a rabbit model of pulmonary emphysema. It was proposed that an altered drive to breathe may be responsible for the sensation of dyspnoea experienced by many patient with emphysema.

1.4 MEASUREMENT AND TESTING OF RESPIRATORY DRIVE

In the present study I intend to measure pattern of breathing and drive to breathe in an animal model of pulmonary emphysema. Drive to breathe may be defined as the rising output of the respiratory central pattern generator. It is my hypothesis that a different drive to breathe in emphysematous animals may explain the shortness of breath experienced by many patients with emphysema. The assessment of respiratory drive must now be considered.

1.4.1 VENTILATION

The most commonly used method to assess the drive to breathe is pulmonary ventilation. Tidal volume is commonly measured directly, either as the volume of gas displaced through the airways (spirometer or pneumotachograph) or as the volume

displaced by the body surface (body plethysmograph). Respiratory frequency is measured directly.

Ventilation is often unsatisfactory for the measurement of drive to breathe, for ventilation is too far removed from the central controller, by a number of variable transformation steps. Abnormalities in neuromuscular transmission or muscular function will affect the controller output-relationship. Changes in the mechanical properties of the lung, airways or chest wall will change respiration for a given respiratory drive (Cherniack & Snidal, 1956; Eldridge & Davis, 1959; Lynne-Davies, Couture & Pengelly, 1971; Milic-Emili & Tyler, 1963). Even an alteration in absolute lung volume (i.e., end-expiratory level) will, by changing the precontraction length of the inspiratory muscle and the radius of curvature of the diaphragm, affect the resultant tidal volume even though the same respiratory drive exists (Evanich, Franco & Lourenco, 1973).

Therefore, other methods have been used to assess neural drive to breathe such as mouth (airway) occlusion pressure, diaphragmatic electromyography (EMG) and phrenic electroneurogram (ENG). The value of these methods in measuring the drive to breathe in pulmonary emphysema will be explored.

1.4.2 AIRWAY OCCLUSION PRESSURE

Airway occlusion pressure is generated at the airway opening when the respiratory muscles contract against the nearly infinite load of an occluded airway. The first systematic use of airway occlusion pressure as an index of "respiratory centre output" was made by Grunstein, Younes & Milic-Emili (1973). Since there is no flow of gas during the occluded inspiration and lung volume does not change (except by a small amount owing to gas compression), the measurement is not influenced by the flow resistance or compliance of the respiratory system.

In conscious subjects the pressure reached after 0.1 sec ($P_{0.1}$) or 0.15 sec ($P_{0.15}$) following airway occlusion are the most commonly used (Milic-Emili, Whitelaw & Grassino, 1981), while in anaesthetised subjects the whole breath occlusion pressure can be measured. In deeply anaesthetised animals, airway occlusion prolongs t_i through the Hering-Breuer reflex, but generally the initial part of the neural discharge is not disturbed (Bradley, 1972; Evanich *et al.*, 1976;

Siafakas, Chang, Bonora, Gautier, Milic-Emili & Duron, 1981; Younes & Riddle, 1981).

Several studies have shown linear relationships between instantaneous integrated diaphragmatic electromyograph (EMG) or phrenic electroneurogram (ENG) and occlusion pressure in anaesthetised cats (Eldridge, 1975; Evanich *et al.*, 1976), dogs (Altose, Aherne & Dunnill, 1976) and conscious human (Lopata, Evanich & Lourenco, 1975). In any given animal the relationship does not vary with alveolar P_{CO_2} (Eldridge, 1975; Evanich *et al.*, 1976) or body temperature (Trippenbach & Milic-Emili, 1977) but is affected by the lung volume at which occlusion is performed being smaller at higher lung volumes (Eldridge & Vaughn, 1977; Evanich *et al.*, 1973; Pengelly, Alderson & Milic-Emili, 1971) where the contraction of inspiratory muscle is less effective (Marshall, 1962) and by chest wall configuration even at the same lung volume (Grassino, Goldman & Mead, 1974). Since pulmonary emphysema is known to change lung volumes, this method is not suitable to measure drive to breathe in the present study.

1.4.3 DIAPHRAGMATIC ELECTROMYOGRAPH (EMG)

Electromyography is the study of the electrical activity generated by muscles during contraction. The EMG of a single motor unit can be recorded using needles electrodes, wires, etc. inserted into the muscle. The action potential has a constant voltage and configuration with a variable frequency of firing. The action potential can be analysed with respect to its configuration (Basmajian & Cross, 1971), area (Lippold, 1952) or frequency of firing (Tanji & Kato, 1972). If the EMG is recorded with surface electrodes, it includes a large population of fibres (global EMG). From that signal, the number of spikes per unit of time can be counted, the average voltage of the spikes measured (Bigland & Lippold, 1954), the area under the EMG computed (Gross, Goldman & Mead, 1976), the frequency spectrum calculated (Gross *et al.*, 1976) or a qualitative visual evaluation performed (Feinstein, Lindegard, Nyman & Wohlfart, 1955).

The integrated surface EMG has been found to bear a linear relationship to the tension developed by the muscle (Milner-Brown & Stein, 1975). Diaphragmatic EMG and/or transdiaphragmatic pressure has been shown to have a linear

relationship to phrenic ENG (Eldridge, 1975; Evanich *et al.*, 1976). This relationship is distorted if the lung resting volume is different from normal, owing in part to force-length relationship of inspiratory muscle (Marshall, 1962; Pengelly *et al.*, 1971). At higher lung volumes (FRC) pressure generated by the diaphragm is less (Siafakas *et al.*, 1981). For the same reasons as given for airway occlusion pressure, diaphragmatic EMG is not suitable to reflect drive to breathe accurately in pulmonary emphysema.

1.4.4 PHRENIC ELECTRONEUROGRAM (ENG)

In the present study, where anaesthetised spontaneously breathing rabbits are used, the opportunity was present of more directly measuring the output of the respiratory centres and hence respiratory drive as phrenic activity. This method was used to assess the drive to breathe, since unlike other methods (ventilation, airway occlusion pressure, diaphragmatic EMG), it has no limitation as an indicator of respiratory drive in pulmonary emphysema.

Since almost all dorsal group medullary inspiratory units have efferent connections to phrenic motoneurons (Berger, 1977; Bianchi, 1971 cited by St. John, 1979; Cohen, Piercey, Gootman & Wolotsky, 1974; Hilaire & Monteau, 1976 cited by Cohen, 1981), the phrenic neurogram has been extensively used as an indicator of the central nervous system respiratory output (Wyman, 1977; Cohen, 1979; Bertrand & Hugelin, 1971; Cohen, 1964; Cohen, 1968).

The phrenic electroneurogram (ENG) records the action potentials of motoneurons in the proximal end of cut spinal nerves and can be measured in two main ways: single fibre recording and whole nerve recording. The former method allows detection of the response of a single motoneuron to various stimuli. In whole nerve recordings made with bipolar electrodes, no single axon spikes are usually detected, the ENG rather representing the summation of many action potentials.

Recording of the phrenic could be obtained from any of the phrenic nerve roots (C3-C6), though C5 has been commonly used. Huszczuk (1971) using the rabbit as the experimental animals, found that electrical signals from either right or left C3 root of the phrenic nerve was related in a linear manner to transpulmonary pressure changes; similar linearity has been found in dogs when recording from C5

root (Bartoli, Cross, Guz, Huszczuk & Jefferies, 1975) and in cat (Siafakas *et al.*, 1981). Hwang & St. John (1993) found that there was no discernible difference between activities recorded from C5 or C6 root on the same side. As it seems there are no differences between the phrenic roots, in the present study any one of the phrenic roots was used.

Phrenic activity can be recorded as a diphasic or monophasic activity. Monophasic activity has been obtained by crushing the proximal end of the phrenic nerve over the peripheral hook of bipolar electrodes (Cohen, 1973). Cohen (1964) recorded simultaneous monophasic and diphasic activity and found that the smoothed signals from both types of recording were proportional to each other over a wide range of phrenic activity levels.

The output of the respiratory controller is distributed to many muscles; diaphragm, intercostal, abdominal, and accessory muscles. The phrenic nerve activity represents the output supplied to the major muscle of inspiration, the diaphragm. There is evidence that with increasing chemical drive there is progressive recruitment of external intercostal and other inspiratory muscles (Campbell, 1955; Corda, Eklund & Euler, 1965). However in anaesthetised animals (Trippenbach & Milic-Emili, 1977; Siafakas *et al.*, 1981) integrated phrenic activity showed a linear relationship to tracheal occlusion pressure and transdiaphragmatic pressure before and after an increase in the drive to breathe. It appears therefore that with increasing respiratory drive the increase in discharge frequency and the recruitment of inspiratory motor units in diaphragmatic and non diaphragmatic muscles occurs in an orderly fashion, such that a fixed relation is preserved (Trippenbach & Milic-Emili, 1977).

In order to gain a better assessment of respiratory drive, phrenic ENG is processed to measure the moving average. This can be measured with a variety of electrical devices such as: a) the leaky integrator - after full wave rectification the raw signal is fed into an RC circuit which provides smoothed voltage tracing (Philbrick Application Manual, 1966), b) the Paynter filter- this is a third order linear phase shift averaging filter (Philbrick Application Manual, 1966), c) digital filtering techniques (Bruce, Goldman & Mead, 1977), d) true integrator with automatic reset (Davies & Wise, 1978), e) electronic computer integration (Wiemer, Kaack & Kezdi, 1975; Feldman & Gautier, 1976). The resultant waveform is an approximate

indicator of average frequency and amplitude of the input potential (Cohen, 1968; Cohen, 1964). In the present study, a method of computer integration of the phrenic activity was evolved

The phrenic integrated activity can be studied in a variety of ways. The rate of rise of the phrenic integral has been used as an index to the rate of rise of central inspiratory activity (Euler, Herrero & Wexler, 1970; Grunstein, Younes & Milic-Emili, 1973; Trippenbach & Milic-Emili, 1977), the peak of the integral being taken as an index to the changes in inspiratory off-switch threshold (Bradley, Euler, Martilla & Ross, 1974; Bradley, Euler, Martilla & Ross, 1975; Euler & Trippenbach, 1976), i.e., the average level of activity at the peak of an inspiratory burst (Cohen, 1964). These indices of the respiratory drive and off switch threshold were also used in the present investigation.

The rate of rise can be quantified by:

- a) calculating the slope of a line drawn on the neurogram from a point at the onset of airflow to the peak height of the neurogram (Pack, Delaney & Fishman, 1981)
- b) the angle between the horizontal and a line joining the beginning and end of phrenic activity (Davies, 1976)
- c) the slope of the line from the onset to 90% of the maximum level of the phrenic ramp (Bongianni, Corda, Fontana & Pantaleo, 1994)
- d) by dividing the peak height by the neural inspiratory duration (St. John & Bartlett, 1979; Hwang & St. John, 1993; Lee, Green & Chiang, 1990).

To facilitate analysis of the phrenic integral in the present study, a computer program was evolved to calculate the respiratory drive (slope of the integral) and the off switch threshold (peak of the integral)

The neural inspiratory duration (t_i) and expiratory duration (t_e) can be directly calculated from the phrenic integral, where t_i is the interval between the initial increase and the levelling off of the phrenic integral, t_e being the interval between the end of one inspiratory duration and the beginning of the next (Davies & Roumy, 1986; Winning & Widdicombe, 1976). Widdicombe & Winning (1974)

compared the t_i and t_E provided from the integral as above with those from the pneumotachograph volume record under a variety of conditions (rebreathing, vagi intact and vagotomy) and found that when t_i of one method is plotted against t_i of the other method the two methods gave a highly linear relationship. t_i and t_E can be indirectly measured from the phrenic integral. Cohen (1968) determined t_i and t_E by using the phrenic neurogram to drive pulses marking the onset of the inspiratory phase (I pulse) and the expiratory phase (E pulse). The phrenic neurogram provides a more direct indication of timing (Clark & Euler, 1972), whilst the flow signal provides an indirect determination of the temporal pattern of the output of the respiratory centre (Gautier, 1973). The flow signal was used in the present investigation to determine t_i , t_E and V_T .

1.5 PRESENT STUDY

Induction of pulmonary emphysema in rats by papain was previously used successfully in this laboratory, and these methods were adopted. However these methods were not directly applicable to rabbits, so a new method of intubation for use with pancreatic elastase had to be evolved.

The presence of emphysema was assessed by measurement of static lung compliance and morphometrically by measurement of mean linear intercept.

The pattern of drive to breathe was assessed by analysis of phrenic motor activity, and the pattern of breathing by the measurement of t_i , t_E and V_T .

The role of pulmonary receptors in changing drive to breathe and pattern of breathing was assessed by using different manoeuvres: a) preSO₂, where all pulmonary receptors are intact b) postSO₂, where slowly adapting pulmonary stretch receptors (SARs) are blocked with sulphur dioxide (SO₂) c) postvagotomy, where all pulmonary receptors are removed by section of the vagus nerves.

By performing these studies, it was hoped to show whether or not there was a changed drive to breathe in the animal models of emphysema, as it is proposed that an altered drive to breathe may contribute to the sensation of dyspnoea seen in emphysematous human subjects.

Chapter 2

2. METHODS

2.1 ANIMALS

Four Sprague Dawley rats (366-410g), four Dutch rabbits (1.5-2.4kg) and four New Zealand white rabbits (2.2-2.3kg) were used for the measurements of conscious pattern of breathing as component of variance by the barometric method.

Ten Sprague Dawley rats (274-340g) and 42 Dutch rabbits (1.8-2.2kg) were used for induction of emphysema and for acute experiments.

Dutch rabbits were purchased from Harlan UK Limited, New Zealand white rabbits from City Farms UK and Sprague Dawley rats from B.K. Universal Limited UK. Although the animals were not bred in pathogen free conditions, they appeared healthy prior to use in this study. After transfer from the suppliers, they were housed in the university animal house and kept in a 12 hour light / dark cycle. A commercial diet and tap water was available ad libitum. Before being used in an experiment animals were allowed at least one full week to acclimatise to their new surroundings and to be checked for gross signs of respiratory tract infection such as encrusted forelimbs or abnormal discharge from eyes and nose (Flecknell, 1987).

2.2 INDUCTION OF EMPHYSEMA

2.2.1 DUTCH RABBITS

Dutch rabbits were rendered emphysematous by endotracheal administration of type IV porcine pancreatic elastase (Sigma E0258).

To avoid the trauma of restraint while insufflating elastase rabbits were initially anaesthetised via the marginal ear vein with an induction dose of propofol (Diprivan, intravenous injection, Zeneca) sufficient to suppress all evidence of consciousness and to allow the mouth to be easily opened. To intubate and dose the rabbit, it was laid prone on a table with its head close to a corner and its body extended close to one edge of the table; so operator and assistant stood facing each other with assistant in front of the rabbit and the operator behind and to one side of the animal. The rabbit's head was tipped back and supported at the angle of the jaw by the assistant who at the same time gently pulled the tongue out of one side of the mouth.

With the operator standing behind the rabbit a Wisconsin laryngoscope with paediatric blade number 1 was inserted and by viewing from behind the dorsal surface of the head the vocal cords could be very well visualised (fig. 2.1). A sterile endotracheal tube O. D. 3.5mm (Portex Ltd., Hythe, Kent, CT21 6JL) or one made from a sterile intravenous white luer cannula (Portex Ltd, UK) was cut to 16cm in length. It contained an introducing wire, lubricated with a water-soluble sterile lubricant (K-Y jelly, Johnson Ltd., Slough, U. K.), and was inserted from this position, even without the necessity of completely obtunding laryngeal reflexes. The tube was inserted gently down the trachea for few centimetre so that the end of the tube was near the carina. This technique is new and has been reported in Laboratory Animals Journal (Davies, Dallak & Moores, 1996).

The introducing wire was then removed and a 240 or 400 units/kg body weight of the elastase dissolved in 1 ml sterile physiological saline was insufflated through the endotracheal tube into the lungs. An additional amount of sterile physiological saline (0.2 ml) was then insufflated to take account of the calculated dead space of the endotracheal tube. The tube was withdrawn. The rabbit was then repeatedly tilted to help the elastase solution reached both upper and lower regions of the lungs. Recovery was rapid and within 5 minutes all rabbits had regained their righting reflexes and were moving calmly about their cage. The dose of elastase chosen was higher than that used by Delpierre et al. (1985). The starting dose was 240 IU/ kg body weight, which was increased to 400 IU/ kg with no mortality. Control animals were not treated with vehicle (saline) as we were specifically interested in disease changes, and anaesthesia and intubation of controls could not be justified under those circumstances. Besides, Damon et al. (1982) found that saline-treated rats have an alveolar structure similar to healthy rats.

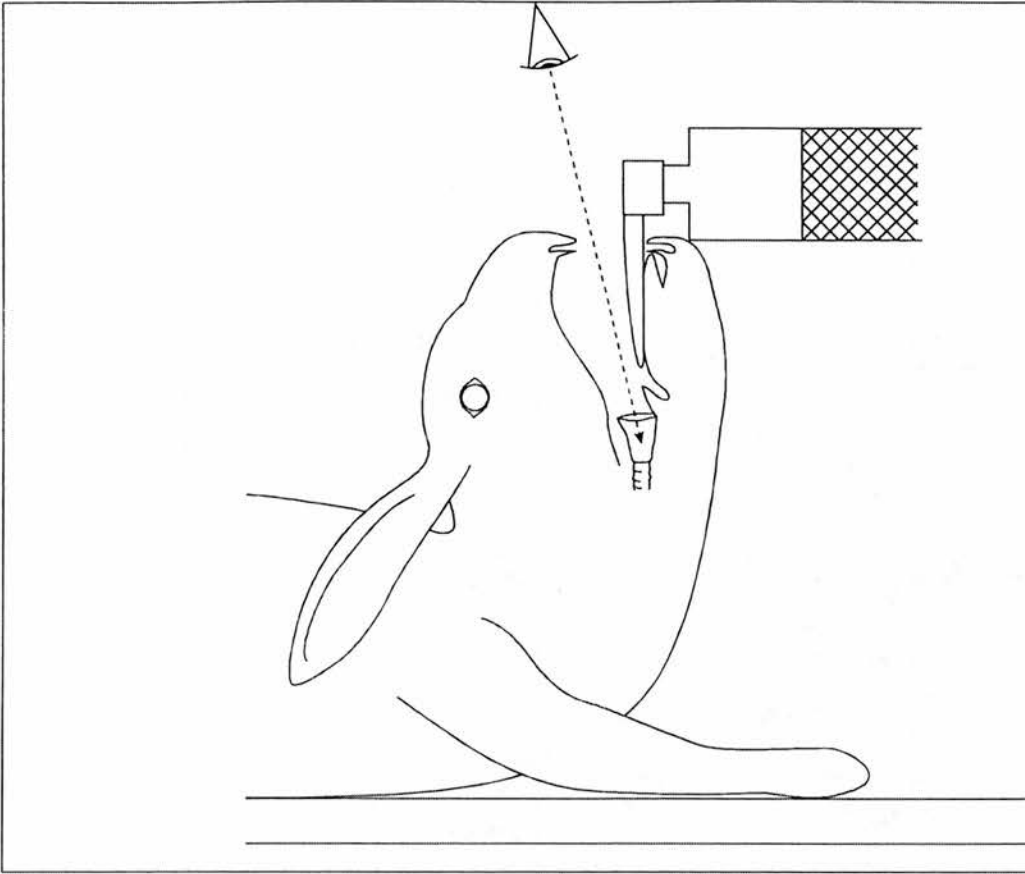


Fig. 2.1 Visualisation of the vocal cords.

A five days course of 1 ml Baytril per day as prophylactic antibiotic was given from the first day of the procedure. 1ml of Baytril contains 50mg enrofloxacin and 30mg n-butyl alcohol as a preservative, which was added to 100ml of drinking water (which is an established veterinary procedure).

Propofol is reported to produce anaesthesia of short duration and light plane in humans however it has a narrow anaesthetic index in rabbits (Aeschbacher & Webb, 1993) and this resulted in a number of anaesthetic deaths. Therefore, the anaesthetic regimen was changed (Flecknell, 1991) after the first 5 rabbits. This consisted of Hypnorm (fentanyl citrate 0.315 mg/ml and fluanisone 10mg/ml) and diazepam. Postoperatively Torbugesic (butorphanol 10 mg/ml and benzethonium chloride 0.1mg/ml) was administered which serve to reverse the respiratory depressant effect of fentanyl.

Hypnorm 0.2ml/kg was given intramuscularly to provide sedation and analgesia, followed about 10 minutes later by diazepam 0.5mg/kg intravenously; intubation was then completed as before. To antagonise the respiratory depressant effect of fentanyl and to provide postoperative analgesia Torbugesic 0.1mg/kg was given intravenously. Complete recovery took approximately 30-40 minutes and during this time, the animal would be lying down in state of neuroleptanesthesia (altered consciousness, breathing deeply and slowly).

2.2.2 RATS

Rats were rendered emphysematous by the endotracheal administration of papain under anaesthesia. The rats were anaesthetised with 4% Halothane in Oxygen in a sealed box connected to a Boyle Fluotec3 anaesthesia machine (Cyprane LTD, UK).

The animals were then held in a vertical position by the scruff of their necks and their mouth kept open with the tongue pulled to one side. A fibre optic light source was used to provide strong direct light and a small metal spatula was used to depress the tongue so that the glottis could be visualised. A plastic endotracheal tube made from a pink luer intravenous cannula (Portex Ltd, UK) cut to 16cm in length and containing an introducing wire, was inserted gently down the trachea for a few centimetres so that the end of the cannula was near the carina. The introducing wire was then removed from the catheter and a dose of 0.05ml/100g body weight of a 240mg/ml solution of papain in normal saline was injected through the catheter. Crude papain was used as a purified preparation of papain has the elastolytic activity removed during the purification process (Snider, Hayes, Franzblau, Kagan, Stone & Korthy, 1974). The volume of papain solution administered to a 500g rat would be 0.25ml of drug solution containing a total of 60mg of papain. To ensure the full dose of papain was given, an additional amount of papain solution was added to the dose to take account of the calculated dead space of the catheter, which was approximately 0.2 ml. The catheter was then removed. The rat was then tilted to help the papain solution reach both the upper and lower regions of the lungs. The rats were then left to recover from the anaesthetic. They usually recovered within 5 minutes of the end of

the procedure. The dose of papain used in my rat model of pulmonary emphysema is the one that was used by Pirie & Davies (1995) and found to produce significant changes consistent with human emphysema and associated with no mortality.

2.2.3 LISTS OF DRUGS

Diazepam (CP Pharmaceuticals Ltd. Wrexham UK.)

Elastase: Type IV porcine pancreatic elastase (Lyophilised powder, one unit will solubilize 1 mg of elastase in 20 minutes at pH 8.8 at 37 °C. Sigma, UK. E0258).

Halothane (May and Baker UK)

Heparin 1250 units/ml (Instrumentation Laboratory)

Hypnorm: 0.315mg/ml fentanyl Citrate/ 10mg/ml fluanisone (Janssen Animal Health)

Crude Papain, No. P- 3375, 2.8 units/mg solid where 1 unit will hydrolyse 1.0 μ mole of N α -benzoyl-L-arginine ethyl ester (BAEE) per minute at pH 6.2 and 25 °C (Sigma UK.). The Papain used was the crude substrate obtained from papaya latex, which was ground to a very fine powder in a pestle and mortar before making up in saline to administer to the animal

Propofol (Zeneca)

Torbugesic: 10mg/ml butorphanol/ 0.1mg/ml benzethonium chloride (Willows Francis Veterinary)

Urethane: ethyl carbamate (Sigma, UK.)

Xylocain 2%: lignocaine hydrochloride (Astra Pharmaceuticals)

2.3 MEASUREMENT OF CONSCIOUS PATTERN OF BREATHING

The major aim of this part of the study was to compare breathing pattern in conscious emphysematous and control rabbits. Having developed a barometric method of measuring the pattern of breathing in rabbits, we took the opportunity of using the method to compare conscious breathing pattern in rabbits with that of rats a species that had been used in previous model of lung disease (Davies & Pack, 1991).

To estimate the minimum number of emphysematous rabbits required to give a statistically valid result for our major aim we need to know the variability of the pattern of breathing in normal rabbits. Variability of pattern of breathing was calculated as components of variance using the commercial analysis program SAS (SAS Institute Inc., SAS Circle, Box 8000 Cary, NC 27512-8000).

For analysis of component of variance, rabbits and rats pattern of breathing was measured in terms of inspiratory duration (t_i , sec), expiratory duration (t_e , sec) and tidal volume (V_T , ml). Rat's conscious pattern of breathing was measured on four separate days when 100 breaths/ day in one run were analysed. The number of breaths for Dutch rabbits was 40/day and that of New Zealand white rabbits was 96/day.

To assess the effect of emphysema, rats conscious pattern of breathing measurement and histological examination were performed before and two weeks after tracheal insutilation of papain (Mansoor et al. 1997). For rabbits histological examination and conscious pattern of breathing measurement were performed before and four weeks after tracheal insutilation of elastase. This period was thought to be necessary so that the rabbits can be calm enough for their conscious pattern of breathing to be measured. For both rats and rabbits pattern of breathing was measured by the barometric method for four consecutive days, 20 breaths each day before and after the induction of emphysema.

2.3.1 BAROMETRIC METHOD

Conscious rabbit's and rat's pattern of breathing in terms of tidal volume V_T , inspiration time t_i and expiration time t_e were measured by using the barometric method of Bartlett & Tenney (1970), which was developed from the method of Drorbaugh & Fenn, (1955), who explained the pressure changes recorded in the chamber on the assumption that when an animal in a closed chamber inhales, the inspired tidal volume is warmed from chamber temperature to body temperature and saturated with water vapour at body temperature. Similarly, on expiration, the tidal volume is cooled to chamber temperature and loses water vapour to the extent necessitated by the cooling. In a closed plethysmograph, this warming and wetting of

the inspire and cooling and drying of the expirate are accompanied by small pressure changes, which can be detected by a sensitive pressure transducer. The barometric method was the method of choice because it requires no tactile contact with the animals. V_T was measured by calibrating the barometric method against a pneumotachograph.

The chamber (volume, 14 l) was made of Perspex, and was cylindrical in shape, of 0.5m length, and inner diameter of 0.19m (Fig. 2.2). One end of the chamber was sealed, the other end was open and could be sealed firmly with a lid. The lid has 4 small openings (3-4mm in diameter): one opening was connected to the reference chamber through a slow leak (see below), the other opening was connected to the pressure transducer (flow meter CS5, Mercury Electronic Ltd. UK) through a plastic tube (0.47m long & 5mm ID), the third opening was directly connected to the calibrating syringe, the fourth opening was the inlet through which flushing air enters the chamber. The flushing air left the chamber through a 1.5cm diameter hole, which during a measurement period was firmly closed with a plastic bung.

An identical chamber was used as a reference chamber which served to make the system independent of barometric pressure changes in the laboratory, such as those caused by opening and closing doors. Both chambers were connected by a slow leak, made from two plastic tubes (9cm long and 0.5cm ID) connected together by an intravenous cannula (11cm long and 1.65mm OD), which served to prevent the pressure recording from drifting off scale due to small temperature fluctuations in the animal chamber. This leak was so slow (half time = 3 sec-see below) that it had virtually no effect on the pressure changes associated with breathing; opening and closing the leak during a measurement period produced no detectable change in the pressure tracing. The half time of the leak was calculated by injecting a known amount of air into the closed animal chamber while the leak tube was clamped. The pressure changes in the chamber were monitored. Once the pressure in the chamber had stabilised, the clamp was released and the time elapsed until the pressure again stabilised was noted.

The animal and reference chamber were connected to a differential pressure transducer (flow meter CS5, Mercury Electronic Ltd. UK) (Fig. 2.3) whose output

was connected to a chart pen recorder (Lectromed Ltd., Recorder type MX 216-87, Channel Islands) and a magnetic tape recorder (7-channels, TEAC-XR30, Japan).

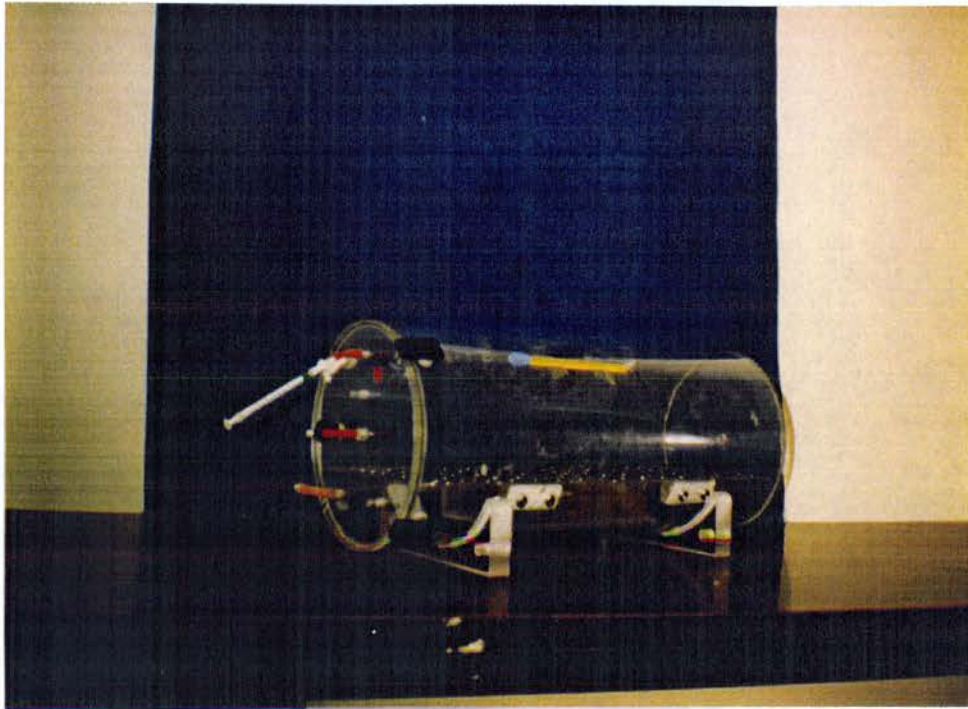


Fig. 2.2 Chamber used to measure ventilation in conscious rats and rabbits.

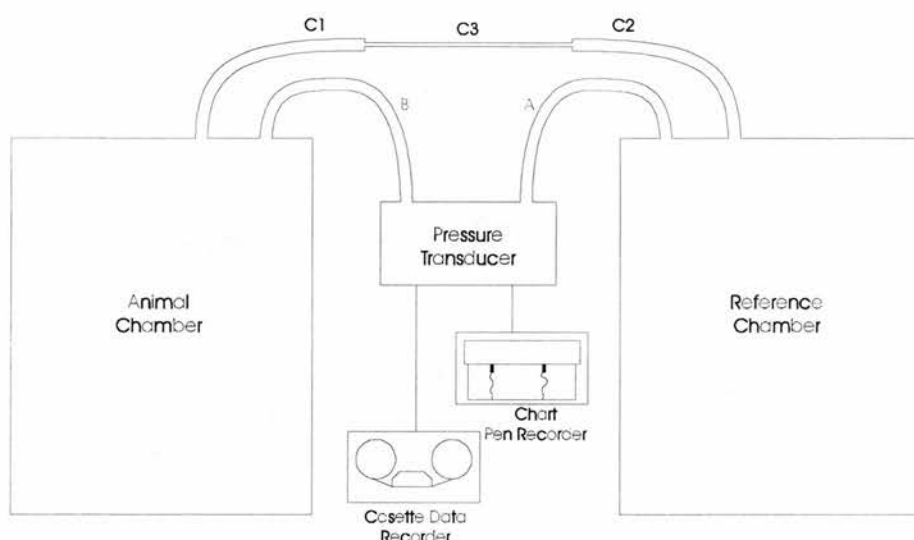


Fig. 2.3 Apparatus used to measure ventilation in conscious animal. The details and procedure are described in the text. A: a rubber tube, 32cm long, 0.5cm ID; B: a rubber tube, 47cm long, 0.5cm ID; C: the leak; C1: a rubber tube, 8.5cm long, 0.5cm ID; C2: a rubber tube, 10.5cm long, 0.5cm ID, C3: an intravenous cannula, 11cm long, 1.65mm ID.

Dynamic response of the system

To validate the barometric method of measuring the conscious pattern of breathing, it was compared directly with a pneumotachograph method using a urethane-anaesthetised rat (Fig. 2.4). With the rat inside the apparatus, the animal chamber was connected to two differential pressure transducers (flow meter CS5, Mercury Electronic Ltd. UK), one measuring pressure changes across a Fleisch pneumotachograph head (Flowhead F10L) connected to a tracheal cannula, the other measuring the pressure changes accompanying animal's breathing in the chamber i.e. the barometric method being validated. Both pressure transducers were connected to the magnetic tape recorder and the chart pen recorder. Breathing variables were measured under eupenic condition. Plotting V_T (barometric) against V_T (pneumotachograph) produced a linear relationship ($r=0.95$) with a slope of 4.74ml.

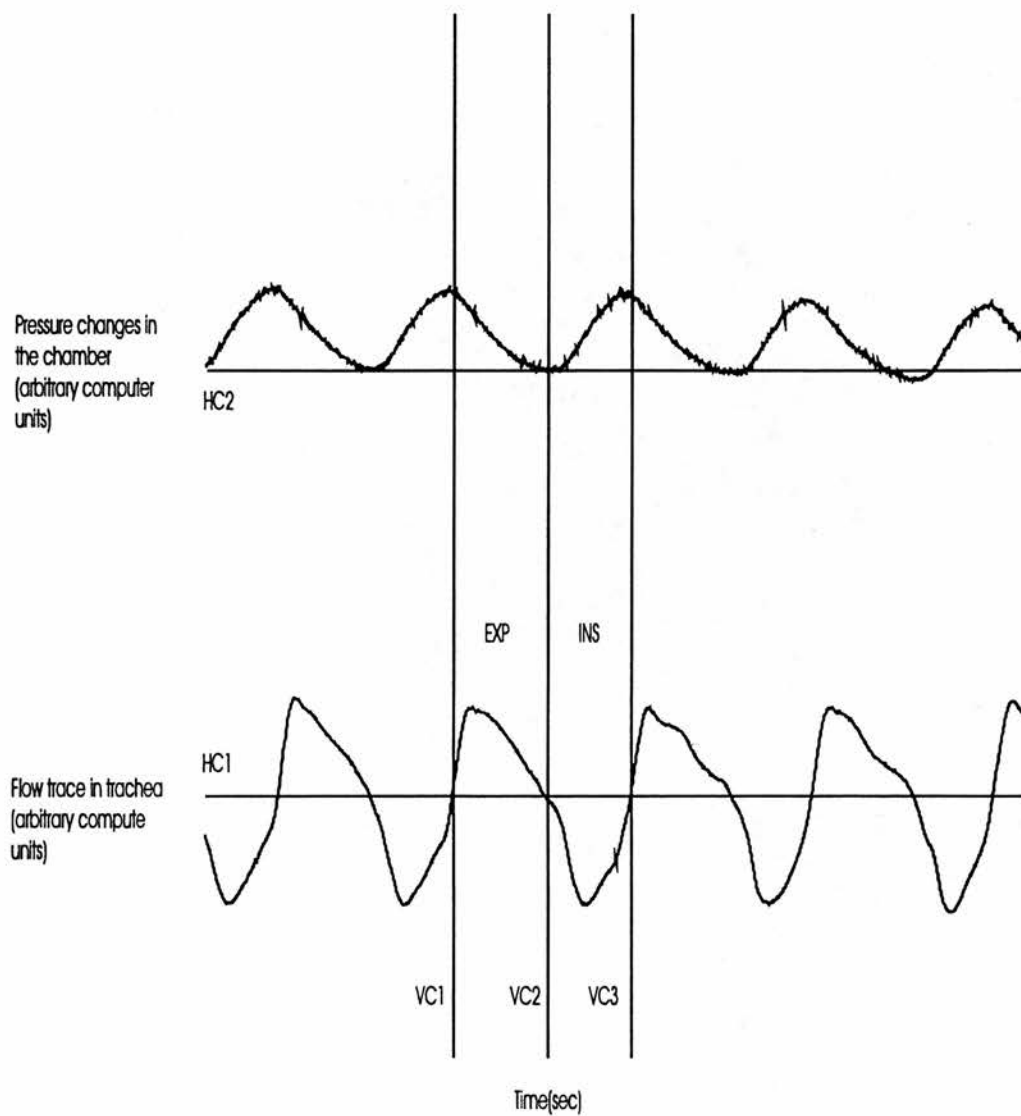


Fig. 2.4 Dynamic response of chamber pressure compared to direct pneumotachograph record of breathing expressed as arbitrary units in computer printout. Horizontal cursor 2 (HC2)= 0 pressure; Horizontal cursor 1(HC1)= 0 flow.

The pressure transducer response time to a step change in pressure was tested. To produce a sudden change in pressure the apparatus shown in fig. 2.5 was used. This consisted of a funnel, whose narrow end was connected through a 3-way tap to the pressure transducer and to a syringe. A light metal plate was gently attached to it's wide end by grease. The pressure within the funnel was decreased by approximately $0.6\text{cmH}_2\text{O}$ by pulling on the syringe's plunger. After the pressure within the funnel had stabilised, the metal plate was quickly removed, producing a sudden change in pressure. This procedure was repeated three times. The time taken for the pressure to drop to zero was calculated from the chart record which was running at 20cm/sec ; this was found to be 0.05 sec. , the delay attributed to the chart recorder's response time was 0.01 sec. This was considered adequate resolution.

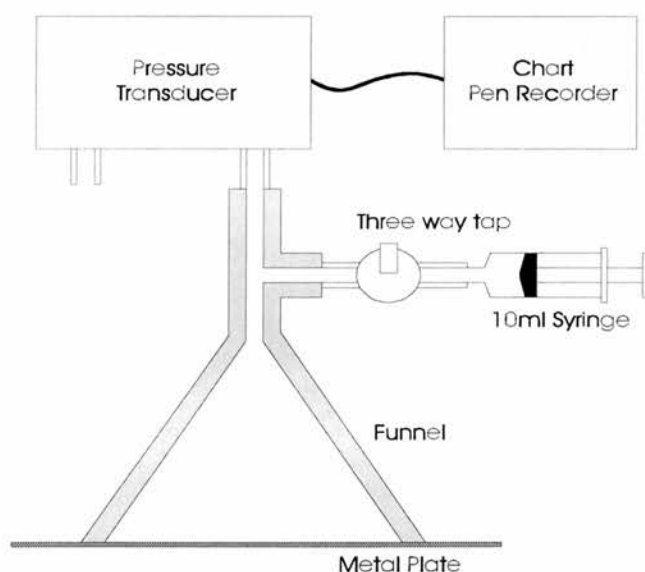


Fig. 2.5 The apparatus used to test the response of the pressure transducer to a sudden change in pressure. See text for details.

Calibration of transducer

In a preliminary series of experiments, pressure changes associated with rabbits' breathing in the chamber were less than $1\text{cmH}_2\text{O}$. Therefore, a sloping water manometer (Fig. 2.6) was used to calibrate the transducer. The pressure transducer

responded linearly ($r=1$) over the pressure range used ($0.1 - 0.5\text{cmH}_2\text{O}$). The rabbits' breathing in the preliminary experiments produced a pressure change of approximately $0.15\text{cmH}_2\text{O}$.

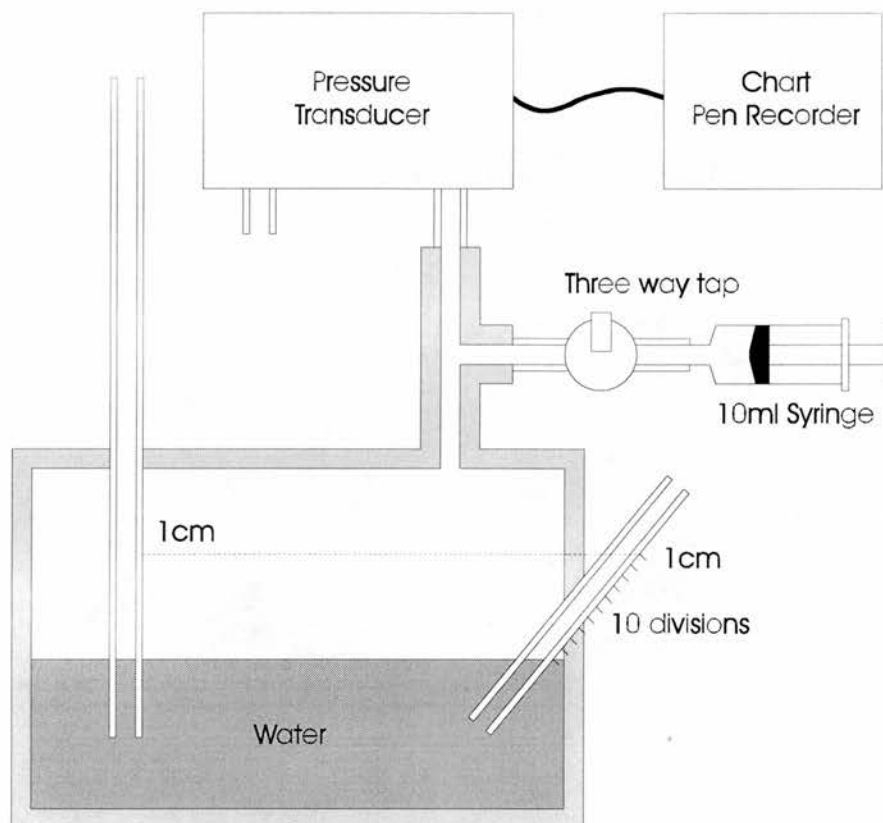


Fig. 2.6 The sloped water manometer used to calibrate the pressure transducer using pressures of less than $1\text{cmH}_2\text{O}$.

Compliance of the system was examined by injecting a known amount of air ($0.5\text{-}4\text{ml}$) using a 5ml syringe, into the chamber. The pressure transducer responded linearly ($r=1$) to these volumes and a slope of 6.4mm/ml was obtained on the chart. The deflection produced by a rabbit's breathing would thus correspond to a 1.6ml change in injected volume.

The frequency response of the whole system was tested using a high frequency ventilating pump (BioScience, Kent, UK) connected to the animal chamber. This pump produces sinusoidal waves of constant amplitude and a wide range of

frequencies. The pressure transducer responded to the frequency range 0.09-12.5 Hz without attenuation of output.

Adiabatic pressure changes

The air-tightness of the chamber and any adiabatic pressure changes which might be present were examined for by injecting a volume of air into the chamber to produce a selected starting pressure and recording the change in pressure. Pressures tested were 1, 3 and 4 cmH₂O. After a 10% reduction in pressure in the first 20 seconds after air injection, the pressure remained unchanged for the rest of the three minutes period during which recording were taken.

Temperature control

The animal and the reference chambers' temperature were kept constant (18 °C) by wrapping the two chambers in cloths soaked in cold water. A fan was used to help cool the chambers and cold water was added regularly to the cloths to keep it constantly wet and cold. The animal chamber's temperature was monitored regularly by placing a thermometer in the chamber through the outlet opening.

2.3.2 PROTOCOL

To familiarise animal with the chamber and personnel, the animal was placed in the chamber for 30-60 min a day for 4-5 days before beginning the experiments. The animal was allowed to move about freely while the chamber was flushed with room air at a rate of 2-4l/min. To allow the animal to become familiar with the situation during recording the inlet and the outlet of the chamber were closed and opened several times each session.

To measure breathing pattern, the animal was placed in the chamber as described above and at the end of familiarisation period of 30-60min, if the animal appeared calm, the inlet and the outlet of the animal chamber were closed, and a 30-60 second record was made of the pressure difference between the animal and the reference chambers. During each measurement period, a small, measured volume of air was injected rapidly into the animal chamber for calibration purposes. The manoeuvre of closing and opening of the inlet and the outlet were performed a few

times before the real measurement was made, to familiarise the animal with the situation and make it finally indifferent to the manoeuvre. The chamber was flushed with air for at least 15 minutes between successive recordings.

Pattern of breathing variables: tidal volume (V_T), inspiration time (t_i) and expiration time (t_e) were measured either directly from chart paper (Fig. 2.7) or through an off-line analysis, where the magnetic tape recorder was connected to an IBM-compatible computer (Gateway2000, p5-120) via model 1401 analogue to digital converter (Cambridge Electronic Design (CED) Ltd, UK). The data were collected using Spike2 software package (CED Ltd, UK). Pattern of breathing variables were then calculated with a script written for the Spike2 analysis package (Appendix 1). They were calculated as follows: inspiration time (t_i) is the time from the zero pressure to the maximum pressure, expiration time (t_e) is the time from the maximum pressure to the zero pressure, tidal volume (V_T) was initially calculated from the equation derived by Drorbaugh and Fenn (1955) and was then accurately calculated by applying the correction factor derived from direct calibration of the barometric method against the pneumotachograph (page 76).

The Drorbaugh and Fenn's equation is:

$$V_T = \frac{P_T}{P_K} \times V_K \times \frac{T_R(P_B - P_C)}{T_R(P_B - P_C) - T_C(P_B - P_R)}$$

The symbols in the equation are defined as follows:

V_T = tidal volume

V_K = the volume of air injected into the animal chamber for calibration

P_T = The pressure deflection associated with each tidal volume

P_K = the pressure deflection associated with injection of the calibrating volume

T_R = body temperature

T_C = the air temperature in the animal chamber

P_B = barometric pressure

P_R = the vapour pressure of water at body temperature, i.e. 47 mmHg

P_c = the vapour pressure of water in the animal chamber (assumed 100% saturate at T_c)

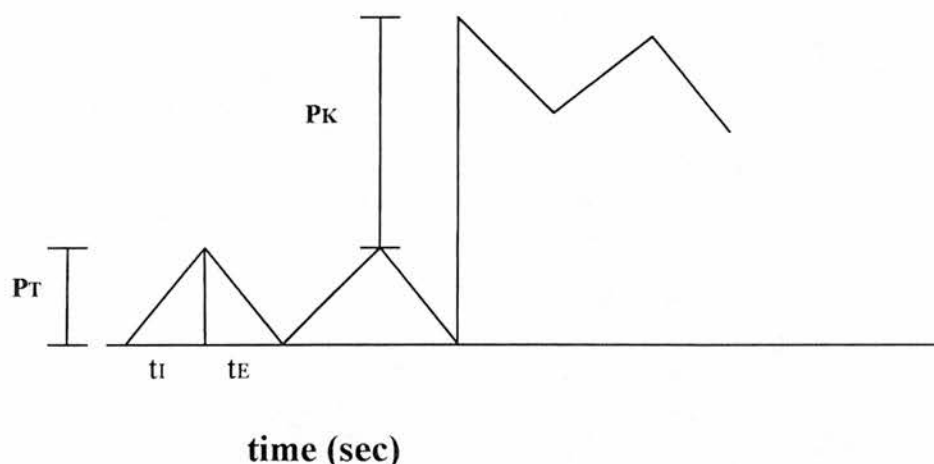


Fig. 2.7 A diagram of a sample measurement of ventilation. The record represents the pressure difference between the animal and the reference chamber. Upward deflections indicate increasing pressure in the animal chamber (inspiration). P_t is the change in pressure associated with each tidal breath. P_k is the pressure resulting from the rapid injection of 2ml of air into the animal chamber.

2.4 POSTMORTEM MEASUREMENTS

2.4.1 LUNG COMPLIANCE MEASUREMENT IN RABBITS AND RATS

Because emphysema in rats was found not to change airway resistance (Boyd, Fisher & Jaeger, 1980) and changes in lung mechanics were used only to confirm presence of the disease, evaluation of lung mechanics in the present study was confined to measuring lung compliance.

Animals used were 20 rats and 67 Dutch rabbits. At the end of the acute experiments and after measurements of conscious pattern of breathing, animals were killed with an overdose of anaesthetic (pentobarbitone) and the lungs were allowed to passively deflate to functional residual capacity (FRC). The tracheal cannula, with the lungs in situ, was connected to a differential pressure transducer (Firnness Control Limited, Bexhill, England) and to a 60ml plastic syringe through a three-way tap. The other port of the transducer was at atmospheric pressure. The transducer was

connected to a chart pen recorder (type MX 216, Lectromed) running at 1mm/sec and at a gain of 5V FSD. The transducer was firstly calibrated using the conventional water manometer (1-20cmH₂O).

To control lung volume history prior to measurements of static compliance (C_{st}) the rabbits' lungs were inflated with 10ml of room air and then allowed to passively deflate, this was repeated four times and the procedure was then repeated using 20, 30, and 40ml volume of air. An inflation volume of 40ml was considered as total lung capacity (TLC) and produced a pressure of 20 - 25cmH₂O.

To produce the deflation volume-pressure curve, lungs were inflated with 40ml air and pressure in the was measured after 20 seconds; 5ml of air was then withdrawn using the syringe connected to the tracheal cannula and pressure was again measured after 20sec. This procedure was repeated until a total of 40ml air had been withdrawn.

Lungs were then actively inflated with 40ml air and allowed to passively deflate; this was repeated in two occasions.

To produce the inflation volume-pressure curve, lungs were inflated with 5ml air and pressure was measured after 20sec; another 5ml was then introduced and pressure was again recorded after 20sec. This procedure was repeated until a total of 40ml had been introduced.

Rats' lung compliance was measured by the same procedure as that used for rabbits, except that the total air volume used was 10ml instead of 40ml and 1ml used in a stepwise fashion instead of 5ml.

The deflation and inflation volume (Y)-pressure (X) curves for each control and emphysematous animal were obtained and the slope of the straight portion of the curve was taken as the static lung compliance (C_{st}) of that particular animal.

2.4.2 METHODS OF ASCERTAINING EMPHYSEMA IN RATS

In this laboratory (Pirie, 1997), blocks from rats' lung were embedded in plastic to verify the presence of emphysema histologically. The same method was used with rat model of pulmonary emphysema used in the present study. However, because

the tungsten-carbide coated knife which cut the sections had become blunt and no spare could be found, it was decided that wax should be used for rabbits.

2.4.2.1 LUNG FIXATION

(i) Fixative

10% formal saline was used as a fixative which was made up as follows: 100ml of technical grade formaldehyde, 900ml tap water and 8.5g of NaCl were mixed together and marble chips added to act as a buffer by neutralising any Formic acid which was produced. The formal saline was stored in an airtight container until required. A fresh solution was made for each experiment.

(ii) Procedure of fixation

Lung fixation was performed by a modification of Heard's method (1958). After the measurement of the static compliance, lungs were carefully removed. The tracheal cannula was connected to a reservoir of 10% formal saline and this fixative was allowed to flow until the 'natural contour' of the lungs were established. The lungs were then floated in a tank of the same fixative and inflation-fixed at a constant pressure of 25cmH₂O for 24 hours. The apparatus used is illustrated in fig. 2.8.

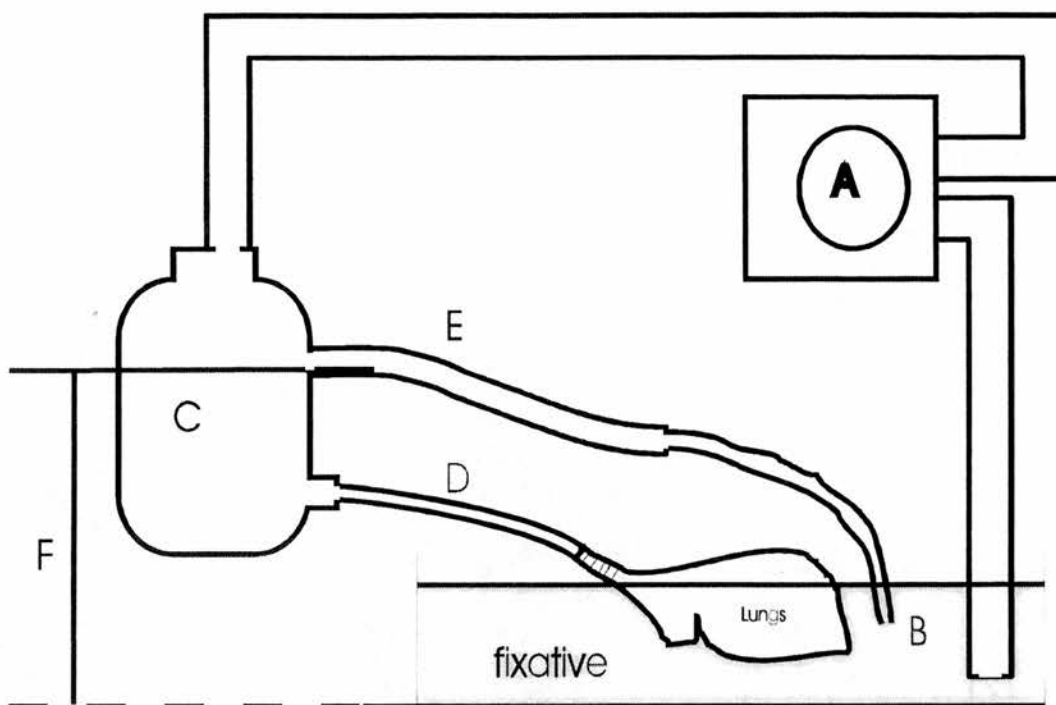


Fig. 2.8 Apparatus used for inflation fixation of lungs at constant hydrostatic pressure of 25 cmH₂O for a period of 24 hours. The pump (A) circulate fixative from the large tank (B) containing the lungs to reservoir (C). The lungs are thus connected to a constant hydrostatic pressure (F) of fixative by tubing (D). The pump (A) runs continuously and overflow runs off via tube (E) to the tank (B).

2.4.2.2 TECHNIQUE OF TRIMMING AND PREPARING BLOCKS OF LUNG FOR EMBEDDING IN PLASTIC

The left lung was used for the microscopic assessment of emphysema as this lung could easily be isolated as it is a single lobe and there was no bias in the production of emphysema by administration of intratracheal papain which had an equal probability of occurring in either side of the lung and to a similar degree. The lung was placed on a cutting board and using a sharp dermatone blade, three mid-sagittal blocks (1x1x1cm): an upper, middle and lower were cut from the isolated left lung.

2.4.2.3 PROCESSING OF FIXED TISSUE FOR EMBEDDING IN PLASTIC (GLYCOLMETHACRYLATE)

(i) Dehydration

The fixed and trimmed blocks were dehydrated through a series of alcohols starting with 70%, then 80%, 95%, and absolute alcohol at room temperature in a vacuum chamber (-400mbar). The blocks remained for two hours in each stage and had two changes of the same alcohol solution, except with 70% alcohol, where more changes were required to cause the blocks to sink which was taken as an absence of air.

(ii) Impregnation and embedding of tissue in plastic resin

(a) Solutions

All the solutions were made in a fume cupboard and were of analar quality

GMA Solution A (impregnator solution):

40ml 2-hydroxyethyl methacrylate

4ml 2-butoxyethanol

0.4gm benzoyl peroxide

(This solution was prepared immediately before use with the peroxide added last)

GMA Solution B (promoter solution):

8ml polyethylene glycol 400

1ml N.N.dimethylaniline

GMA polymerising solution:

42 parts solution A

1 part solution B

(The solution was mixed thoroughly and used immediately)

Gelatine:

Gelatine 1g

chromic potassium sulphate 0.1g

distilled water 200ml

(the gelatine was dissolved first then chromic potassium sulphate was added)

(b) Technique of impregnation and embedding in plastic

The embedding procedure involving the polymerisation reaction was carried out in the fume cupboard

Blocks were placed in GMA solution A and left for 24 hours under vacuum (-400mbar). The blocks were changed once more into fresh solution A and left for a further 24 hours under vacuum.

For each batch of lungs that were embedded, a test polymerisation reaction was carried out to check that the polymerisation occurred controllably. This was important because if the reaction occurred too quickly, bubbles would form in the plastic. Sufficient solution for the whole batch to be processed was then made up.

The blocks were placed in plastic embedding moulds (Polysciences Inc, Warrington, UK) with the lateral surface placed at the bottom of the mould. The polymerisation solution was poured over them to three quarter fill the moulds. With a pair of round ended plastic forceps, the blocks were gently pushed to the bottom of the mould. The polymerisation usually took approximately 10-15 minutes before the solution started to become tacky and thicken, depending on the temperature of the surroundings.

Once the reaction was under way and heat was being produced, the moulds were transferred on to ice to dissipate the heat to prevent gas bubbles forming in the blocks.

When the blocks had become hard, they were placed in a 60°C oven overnight to fully harden. They were then removed from the moulds and trimmed with a hacksaw to a convenient size for sectioning.

2.4.2.4 PRODUCTION OF THIN PLASTIC SECTIONS

All the blocks were trimmed and oriented using a Reichert-Jung Autocut Microtome. A tungsten-carbide coated knife was used on the microtome to cut the sections. Thick sections were cut from the blocks until lung tissue started to appear in the sections and trimming continued until a complete 1x1cm section of lung was produced.

The microtome was then set to cut at 6µm and nine sections were cut and collected. These sections were floated out on a hot water bath (60°C) and individually guided on to gelatine coated microscope slides. The slides were then left for 2-8 hours on a hot plate (60 °C) to dry.

2.4.2.5 STAINING PROCEDURE

(i) Reagents and solutions

Harris's Haematoxylin

Haematoxylin 1g

absolute alcohol 10ml

ammonium or Potassium Aluminium 20g

distilled water 200ml

mercuric acid 0.5g

Scots tap water substitute (S. T. W. S.)

sodium bicarbonate 3.5g

magnesium sulphate 20g

tap water 1000ml

DPX: is a mixture of distrene, a plasticizer and xylene

(ii) Staining and mounting

1. The sections (on slides) were stained in haematoxylin for 3-5 minutes
2. They were then washed well in running tap water for 2-3 minutes, left in S. T. W. S. for 3 minutes, leaving the nuclei stained blue. The section was examined microscopically at this stage to confirm a sufficient degree of staining. If insufficient, sections were returned to stain
3. Sections were then stained in 1% aqueous eosin for 1-3 minutes
4. Surplus stain was washed off using tap water
5. Sections left in saturated Potassium Aluminium sulphate for 2 minutes to bind eosin in the tissue
6. Sections washed well in running tap water for 2 minutes
7. Sections dried on hot plate (60 °C) for 2 hours
8. Sections covered with DPX and then mounted using glass cover-slips.

2.4.3 METHODS OF ASCERTAINING EMPHYSEMA IN RABBITS

2.4.3.1 LUNG FIXATION AND VOLUME MEASUREMENT

(i) Fixative

(ii) Procedure of fixation

Before fixation, fresh lung weight was measured. Fixative and procedure of fixation were the same as for rats (2.4.2.1)

(iii) Volume measurement

Following fixation, the volume of the lungs was measured by water displacement (Aherne & Dunnill, 1982) using a polythene measuring jug fitted with a discharge siphon (polythene tube, 0.5cm ID) (Fig. 2.9). Five repetitions of this volume measurement were completed to yield an acceptable mean value and standard error (Aherne & Dunnill, 1982).

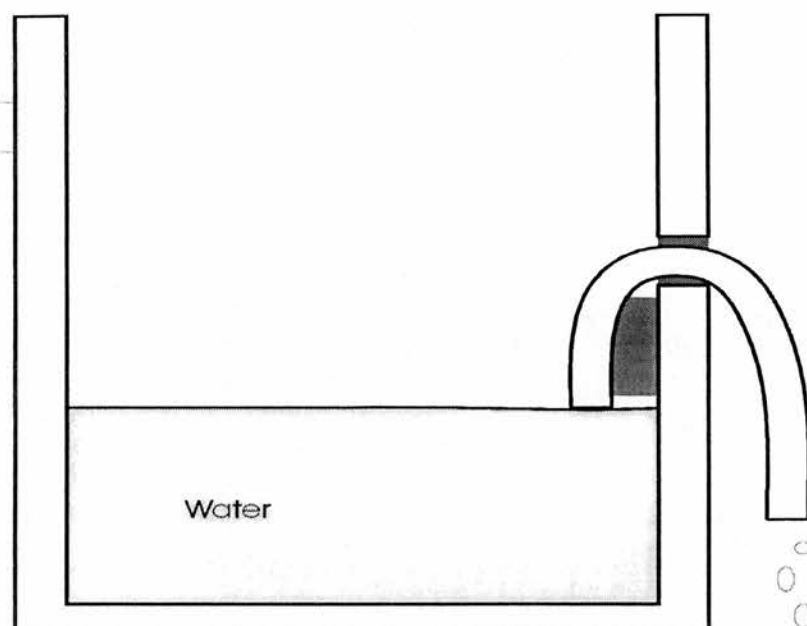


Fig. 2.9 A polythene measuring jug, with discharge siphon, for estimating the volume of the lung. The fluid level is shown at the moment at which the discharge of displaced water is cut off by the siphon.

2.4.3.2 TECHNIQUE OF TRIMMING AND PREPARING BLOCKS OF LUNG FOR EMBEDDING IN PARAFFIN WAX

The right and left lungs were used for the microscopic assessment of emphysema. One mid-sagittal block (1x1x1cm) was cut from each lobe of both lungs i.e. two blocks from the cranial lobes of both lungs, one from the middle lobe of the right lung and two from the lower lobes of both lungs.

2.4.3.3 PROCESSING OF FIXED TISSUE FOR EMBEDDING IN PARAFFIN WAX

(i) Dehydration

The technique used for dehydration of the blocks was the same as that used for plastic (2.4.2.3).

(ii) Impregnation and embedding of blocks in paraffin wax

(a) Reagents

Paraffin wax: Paraplast is a compound of purified paraffin and plastic polymers of regulated molecular weight. Double filtered-free of foreign contaminants. It contains dimethyl sulphoxide for rapid tissue infiltration. melting point 55-57 °C (Sherwood Medical, Athy, Ireland).

Toluene (BDH,UK)

cedarwood oil (BDH,UK)

(b) Technique of impregnation and embedding in paraffin wax

Blocks were placed in a universal bottle full of cedarwood oil and placed on a rotator (1-2 RPM) overnight, if they were not due to be processed immediately after the absolute alcohol stage.

After immersion in cedarwood oil overnight or if processed immediately after the absolute alcohol stage, blocks were placed in toluene on the rotator until they were clear (transparent). This usually took 4 hours.

Blocks were then placed in plastic embedding moulds (Polysciences Inc, Warrington, UK) full of pure molten wax. With a pair of round ended forceps the blocks were gently pushed to the bottom of the moulds, which were then placed in a vacuum oven (-400mbar, 60 °C) for 2 hours.

When removed from the vacuum oven, the wax started to harden and the moulds were placed in a cold water bath (20 °C) to cool the process and therefore to reduce air bubble formation.

Blocks were left in water for 2 hours and then removed from the moulds and trimmed with a hacksaw to a convenient size for sectioning

2.4.3.4 PRODUCTION OF THIN PARAFFIN WAX SECTIONS

All blocks were trimmed and oriented using a rotatory microtome for paraffin wax. Disposable blades (Wilkinson Sword, England) were used to cut the sections. Thick sections were cut from the blocks until lung tissue started to appear in the sections and trimming continued until a complete 1x1cm section of lung was produced. The microtome was then set to cut at 6 μ m, following which nine sections were cut and collected. These sections were floated out onto a hot (50°C) water bath and individually guided on to gelatine coated microscope slides. The slides were then left in an oven at 37°C overnight.

2.4.3.5 STAINING PROCEDURE

(i) Reagents and solutions

Reagents and solutions were the same as those used for plastic (2.4.2.5)

(ii) Staining and mounting

1. Sections (on slides) were dewaxed in xylene for 10 minutes
2. They were then dehydrated in descending alcohol solutions, first in absolute alcohol, then 90%, 80% and 70% alcohol, each for 1 minute. They were then quickly washed in water to remove xylene
3. Sections were stained in haematoxylin for 3-5 minutes
4. Sections were washed well in running tap water for 2-3 minutes, and left in S. T. W. S. for 3 minutes to leave the nuclei stained blue. The section was examined microscopically at this stage to confirm a sufficient degree of staining. If insufficient, sections were returned to stain
5. Excess stain was removed by decolorizing (differentiating) in 1% acid alcohol for few seconds and then stage 4 was repeated to regain the blue colour. The stain was again checked microscopically until proficiency in naked-eye control of decolorization has been gained by experience with stain and tissue
6. Stained in 1% aqueous eosin for 1-3 minutes
7. Surplus stain was washed off in water

8. The sections were left in saturated Potassium Aluminium Sulphate for 2 minutes to hold eosin in the tissue
9. Sections washed well in running tap water for 2 minutes
10. Sections dehydrated in ascending alcohol solutions (70%, 80%, 90% and finally absolute alcohol) one minute in each solution
11. Sections were cleared in Xylene for 3 minutes, covered with DPX and mounted using glass cover-slips.

2.4.4 ASSESSMENT OF EMPHYSEMA IN RABBITS AND RATS

Emphysema was quantitated by using the widely accepted method of measuring the mean linear intercept (**Lm**) (Dunnill, 1962). The mean linear intercept, which is the average distance between alveolar walls, was measured for two sections from each block. The stained lung section slides were examined under light microscope (x20 objective and x10 eyepiece) whose eyepiece was fitted with crossed hair lines of equal and known length. The two hair lines are at right angles to each other to compensate for any deformation that may occur to the section during cutting and mounting (Dunnill, 1962).

Ten fields per section were randomly chosen by blind random displacement of the mechanical stage without observation through the tube (Weibel, 1963). To eliminate bias, the horizontal and the vertical stage micrometers were set each time at an integer millimetre position. For each field, the number of points at which the lines on the cross hair cut through the alveolar walls were counted. A cut through an alveolar wall counts as a single intercept, a cut into a blood vessel wall counts as half an interval, as does a cut out of a blood vessel wall. In this way the number of intercepts were counted on both the vertical and the horizontal hair line for each field, **m**. The mean linear intercepts (**Lm**) for each field was then calculated from **m**, the number of intercepts, **L**, the length of the traverses and **N**, the number of times the traverses are placed on the lung, i.e., in this case twice for each field: $Lm = N.L/m$. Microscopic fields with large bronchi or/and blood vessels >2mm in diameter were avoided.

Lm was measured for two sections for each block. Lm was calculated for each field and the mean of 20 fields for each block was obtained.

2.5 ACUTE EXPERIMENTS

2.5.1 ANIMALS

Dutch rabbits of either sex weighing 1.8-2.2kg were used. 35 emphysematous and 25 normal rabbits were used in the study.

To remove the hypoxic drive to breathe, rabbits were made to breathe 100% oxygen (flow, 3l/min) by connecting an O₂ cylinder to the tracheal cannula through a three-way connector, the excess escaping to atmosphere.

2.5.2 ANAESTHESIA

Rabbits are easily stressed and the combined effect of stress and anaesthesia can result in cardiac and respiratory arrest, and so a few minutes were spent to calm the rabbit before starting anaesthesia.

The fur around the marginal ear vein was shaved and a butterfly needle (Microflex infusion set 0.5mm/G.25) was inserted into the vein. A urethane solution (8ml/kg body weight) was then slowly infused into the animal.

Urethane (ethyl carbamate, Sigma, UK) solution was made by dissolving 25g of urethane in 100ml normal saline. Urethane produces a stable, long lasting anaesthesia with minimal depression of circulatory or respiratory system (Buelke-Sam, Holson, Bazare & Young, 1978). Approximately every 30 minutes, the level of anaesthesia was evaluated by testing the palpebral reflex (blinking when the edge of the eyelid is slightly touched), the reaction to ear pinching (shaking of the head or vocalisation) and the pedal withdrawal reflex (limb withdrawal or muscles twitch when one limb was extended and the web of skin between the toes was firmly pinched). But in fact with urethane there was no apparent change in depth of anaesthesia throughout the experiment.

Rabbits were placed in supine position in a v-shaped support, which was lined with a homoeothermic heating blanket (BioScience, Sheerness, Kent, UK), whose

controlling thermistor probe was inserted into the animals rectum. The blanket was wrapped around the animal with bubble packing and the animal's temperature was maintained at 39°C.

2.5.3 TRACHEAL CANNULATION AND AIR FLOW MEASUREMENT

A mid line incision was made in the neck. The trachea was located and then incised with a scalpel blade just below the larynx between the 2nd and 3rd cartilaginous rings. The cut was wiped free of blood using cotton wool (to prevent any obstruction) and a tracheal cannula (4.5mm OD and 4mm ID) made from the cover of a hypodermic needle (19Gx1.2inches) was inserted into the trachea. The cannula was secured in place by tying firmly with silk thread. Care was taken to avoid damage the recurrent laryngeal nerve.

To measure air flow the tracheal cannula was connected to a Fleisch pneumotachograph head, which was in turn connected to a commercial flow meter (flow meter CS5, Mercury Electronic Ltd. UK). Flow was integrated electronically to give volume. The pneumotachograph was calibrated at the beginning of each experiment with 10, 20 and 30ml of air using a high accuracy rotameter.

2.5.4 FEMORAL ARTERY AND VEIN CANNULATION AND RECORDING OF ARTERIAL BLOOD GASES AND BLOOD PRESSURE

An incision was made in the left groin, the femoral vein and artery were located and cannulated using intravenous cannulae (white luer OD 1.34mm and pink OD 1.02mm (Portex Ltd. England) respectively).

The venous line was used for administration of drugs if required. The arterial line was connected to a three-way tap to which two 1ml plastic syringes were connected. The arterial cannula and the 1ml syringes were initially flushed with balanced heparin solution at a concentration of 1250 IU/ml (Instrumentation Laboratory, Italy). The dead spaces of the syringes were filled slowly (so to leave no air bubbles) with the balanced heparin, to give a final heparin concentration of 20-50 IU/ml when the syringes were filled with the blood samples.

To obtain an arterial sample anaerobically, a 1ml sample of blood was first withdrawn slowly into one of the 1ml syringes connected to the three-way tap, to flush out the dead space; this was replaced into the animal after finishing sampling. A 1ml sample of blood was then withdrawn into the other syringe. A total of three samples of 1.0 ml of blood were taken. The same procedure was performed after administration of 100% O₂ to the animals for 2 minutes. Each 1ml syringe was capped immediately after sampling, then blood and anticoagulant were mixed well by rolling and inversion of the syringe for at least 20sec. The six syringes were put in a container full of ice and were taken for blood gas analysis (Paco₂ and Pao₂) using a Ciba-Corning Diagnostic Ltd UK 238pH blood gas analyser. The blood samples were analysed within a few minutes of being taken.

Blood pressure was monitored by connecting a pressure transducer (Bell & Howell) to the arterial cannula. The pressure transducer was calibrated using a mercury manometer.

2.5.5 PHRENIC NERVE RECORDING

To locate the left phrenic nerve, the ipsilateral sternomastoid muscle was cut, the two ends were clamped by haemostatic forceps and retracted. The left phrenic nerve was identified and one of the upper roots of the nerve was carefully dissected from the surrounding tissue using micro-dissecting forceps and scissors. The root was then cut, the central cut end was freed from surrounding tissue for about 5mm and placed in a copper tray full of liquid paraffin. Using micro-dissecting instruments and under direct vision (Weis Dissecting Microscope) the central end was desheathed and mounted on a pair of silver electrodes that were held by a micro-manipulator (Prior, England). The nerve was always immersed in the liquid paraffin to protect it from drying.

The signal from the electrodes was passed through a high impedance unity gain headstage (NL100 Neurolog system, Digitimer Ltd, England), then differentially amplified (gain 5k) by an AC preamplifier (NL104 Neurolog system, Digitimer Ltd, England), and bandpass filtered (5Hz - 50kHz) with a neurolog filter (NL125 Neurolog system, Digitimer Ltd, England). The signal was also audio amplified

(NL120 Neurolog system, Digitimer Ltd, England) and passed to a speaker. The resulting amplified and filtered signal was displayed on an oscilloscope (HM203-7, Hameg).

Variables: flow, volume, end-tidal carbon dioxide, and phrenic signal were all recorded on video magnetic tape (E-HG240, TDK) by a cassette data recorder (XR 30, TEAC CORP, Japan). On playback, the flow and the phrenic recorded signals were amplified by an AC-DC amplifier (NL106 Neurolog system, Digitimer Ltd, England) and passed through a 1401 analogue to digital converter (Cambridge Electronic Design Ltd, UK) to a computer (Gateway 2000, P5-120) using the "spike2" software package (Cambridge Electronic Design Ltd, UK) where flow was sampled at a rate of 101Hz and phrenic signal at 3333Hz. Data was stored on the hard disk for further analysis.

2.5.6 ACCELERATED BREATHING AND END-TIDAL CARBON DIOXIDE (CO₂) MEASUREMENT

The arterial partial pressure of carbon dioxide was increased by causing the rabbits to breathe 4% carbon dioxide in O₂ for two minutes and then 6% CO₂ in O₂ for a further two minutes. The carbon dioxide mixtures were prepared by mixing commercial carbon dioxide with 100% oxygen and the resulting mixture was blown past the free end of the pneumotachograph head through a three-way connector (see experimental set up, fig. 2.11).

The carbon dioxide in the expired air was monitored by an infrared gas analyser (Model no. 038, P.K. Morgan Ltd, Rainham, Kent, England), sampling at 300ml/min from the external side of the pneumotachograph head. The analyser was calibrated at the beginning, the middle and the end of the experiment using a gas mixture consisting of 5% CO₂, 12% O₂ and balance 83% N₂. The differences in the amount of O₂ i.e. 12% used during calibration of CO₂ analyser and that of inspired air i.e. 100% O₂ was said to give a 5% positive error of the actual reading (P.K. Morgan Ltd).

2.5.7 INFLATION AND DEFLATION STEPS

The rabbit's response to steps of maintained lung inflation and deflation (5 cmH₂O & 10cmH₂O) were recorded. Alternate steps of inflation and deflation were repeated four times. The rabbits were allowed to take at least 20 breaths between steps. The 5cmH₂O inflation pressure was maintained for six consecutive breaths from the onset of the pressure, while the 10cmH₂O inflation pressure was maintained until the animal took a spontaneous inspiration. The deflation pressures (5 & 10cmH₂O) were maintained for a period of ten consecutive breaths. Inflation was manually synchronised with beginning of inspiration and deflation was manually synchronised with beginning of expiration by listening to phrenic activity on the audio amplifier, and this was later verified by looking at the flow signal on the computer when analysing the data.

Inflation and deflation of the lungs with the required pressures were achieved using the apparatus overleaf (Fig. 2.10).

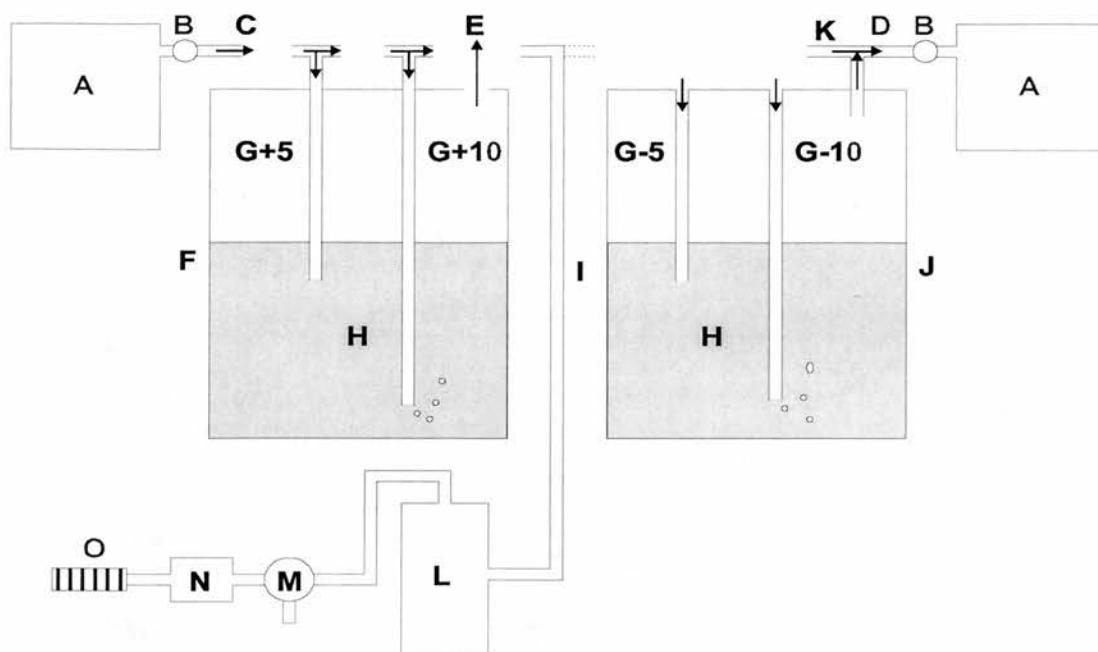


Fig. 2.10 Apparatus used to inflate and deflate the animal's lungs with $\pm 5\text{cmH}_2\text{O}$ and $\pm 10\text{cmH}_2\text{O}$. See text for details.

A: an air pump and a vacuum (Model DOA-P104-BN. MFG.CORP. USA)

B: an adjuster, to adjust the amount of air leaving the pump or coming to the vacuum

C: rubber tube(1cmID), which connects the air pump to the appropriate polythene tube in cylinder(F)

D: rubber tube, connects the air vacuum to the connector (K) in cylinder (J)

E: opening to atmosphere

F: graduated plastic cylinder (44cm long&6.5cm in diameter)

G: four polythene tubes (0.5cmID): G+5 & G-5 have their lower ends 5cm below the water surface. G+10 & G-10 have their lower ends 10cm below the water surface

H: water

I: rubber tube that connects the air drum (10l) to the cylinder (F) or to cylinder (J)

J: graduated plastic cylinder (44cm long&6.5cm in diameter)

K: three-ways connector

L: an air drum (10L)

M: solenoid valve

N: pneumotachograph head

O: tracheal cannula and trachea

To inflate the lungs with 5cmH₂O pressure, tubes (C) and (I) were connected to (G+5) then the pump adjusted (B) till air bubbles just appeared at the lower end of the tube. The solenoid valve (M) was then connected to the pneumotachograph head (N) and triggered manually to rapidly connect the tracheal cannula (O) from the atmosphere to the 10l drum (L). To inflate with 10cmH₂O, tubes (C) and (I) were connected to (G+10) and the procedure repeated as with 5cmH₂O.

To deflate with 5cmH₂O, tubes (D) and (I) were connected to the three-way connector (J) and the upper end of (G-10) sealed. The pump leak was adjusted (B) until air bubbles just appeared at the lower end of (G-5). The solenoid valve (M) was connected to the pneumotachograph head (N) and triggered manually. To deflate with 10cmH₂O, tube (G-5) was sealed and the procedure repeated as with 5cmH₂O.

2.5.8 SLOWLY ADAPTING PULMONARY STRETCH RECEPTOR (SAR) BLOCKADE WITH SULPHUR DIOXIDE (SO₂)

The second part of each experiment was carried out after the animal had breathed 200 parts per million (ppm) SO₂ (Liquefied Sulphur Dioxide, BDH Chemicals Ltd, Poole, England) in air for 30 minutes to block the slowly adapting pulmonary stretch receptors.

The SO₂ mixture was made in a polyethylene Douglas bag (volume, 150 litres) which had been slowly filled, three quarters full (113 litres) by an air pump (Royal Appliance MFG, USA) through a three-way connector. 150ml gaseous SO₂ was injected into the bag using a 50ml glass syringe. This was found by experience to provide the required concentration as measured using a Dräger Tube CH 24201 (Drägerwerk Aktiengesellschaft, Germany). Significant deviation from 200ppm were adjusted by addition of air or SO₂. A plastic tube (0.8mm ID) was then connected the Douglas bag to the animal's tracheal tube through a three-way connector. The pressure in the bag was kept positive by putting a weight on it.

Receptor block was judged to be complete when the Hering-Breuer inflation reflex in response to lung inflation by a pressure of 10cmH₂O, was abolished.

2.5.9 BILATERAL VAGOTOMY

The third part of each experiment was carried out 10 minutes after bilateral vagotomy. Both vagi were exposed in the mid-cervical region and loops of silk thread were placed around them to facilitate identification and sectioning. At the beginning of the third part of the experiment, the vagi were cut using a pair of fine scissors.

2.5.10 EXPERIMENTAL PROTOCOL

The complete experimental set-up is shown below

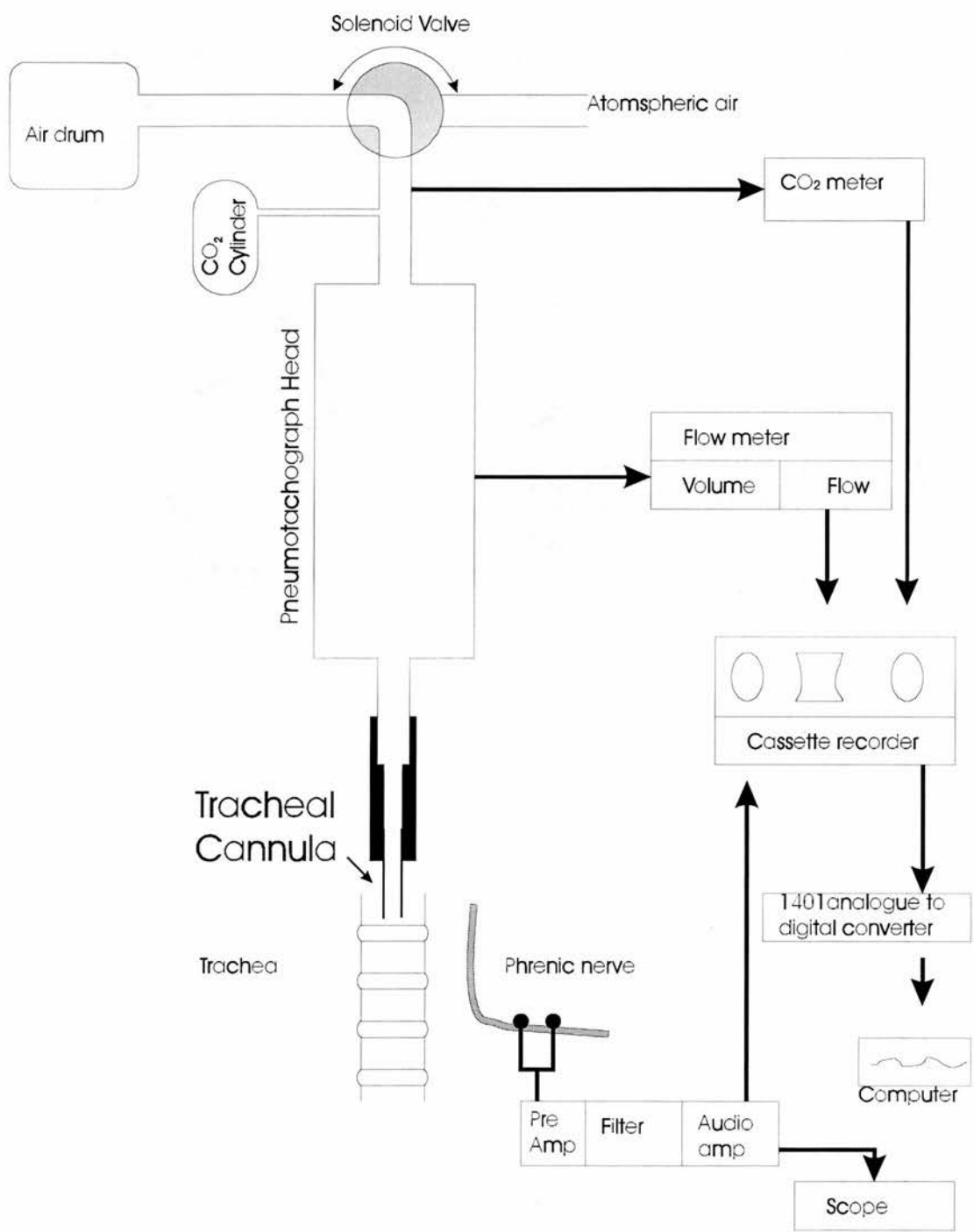


Fig. 2.11 Schematic diagram of the experimental set-up. See text for details.

There were three different parts within each experiment. 1st part is preSO₂, where all pulmonary receptors are intact. 2nd part is postSO₂, where SARs were blocked with SO₂. 3rd part is postvagotomy, where all pulmonary receptors were removed by bilateral vagotomy (table 2.1).

In 1st and 2nd parts there were three stages that have been analysed: control breathing, breathing against positive and negative pressure, and breathing accelerated with 4&6% CO₂. In 3rd part there were two stages: control breathing and accelerated breathing with 4 & 6% CO₂.

Table 2.1 Protocol used with each experiment. See text for details.

Parts	Stages
pre SO ₂	1) control breathing
	2) inflation & deflation steps
	3) 4&6% CO ₂
postSO ₂	1) control breathing
	2) inflation & deflation steps
	3) 4&6% CO ₂
postvagotomy	1) control breathing
	2) 4&6% CO ₂

2.5.11 DATA ANALYSIS

Each breath was analysed by a script written for the “spike2” analysis package (appendix 1) as shown in Figures 2.12 & 2.13.

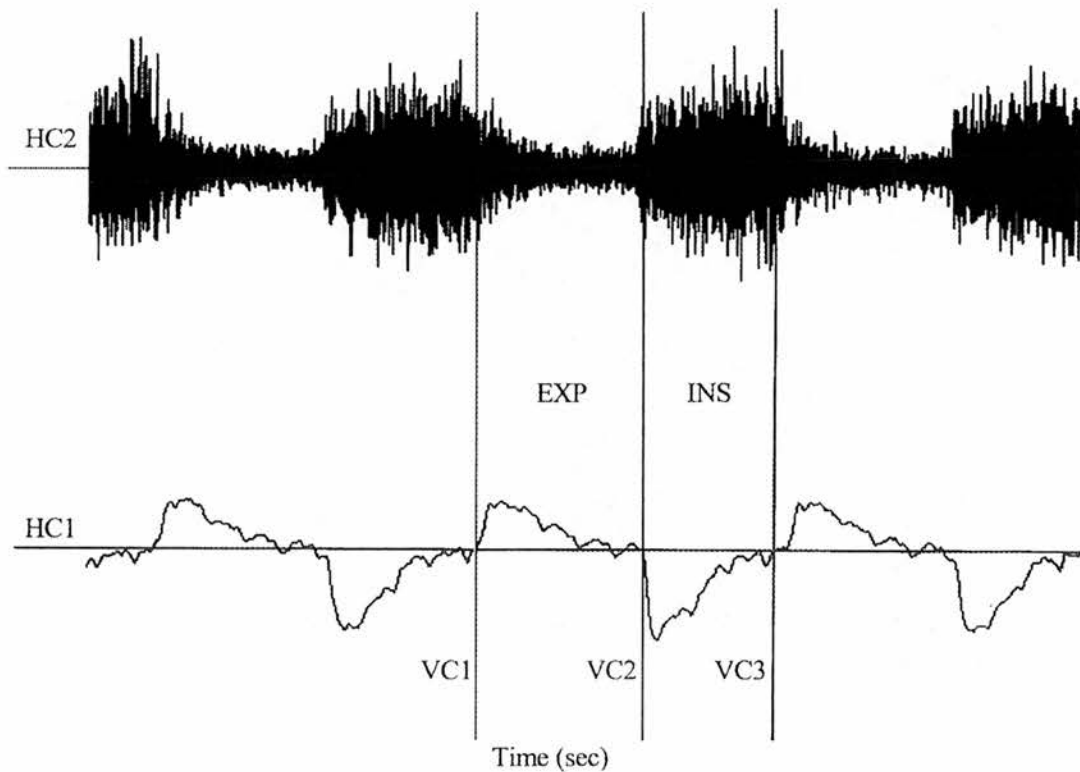


Fig. 2.12 Data as displayed on the computer screen during analysis with the script written for the “spike2” analysis package. EXP: expiratory phase, INS: inspiratory phase, HC: horizontal cursor, VC: vertical cursor. Upper trace: phrenic activity. Lower trace: flow.

To analyse a breath, vertical cursor1 (VC1) was set manually at the beginning of expiration using the flow trace, VC2 was set at the end of the expiration/beginning of inspiration and VC3 was set at the end of inspiration. Horizontal cursor1 (HC1) was set at zero flow and HC2 was set at the middle of phrenic activity during expiration (Fig. 2.12).

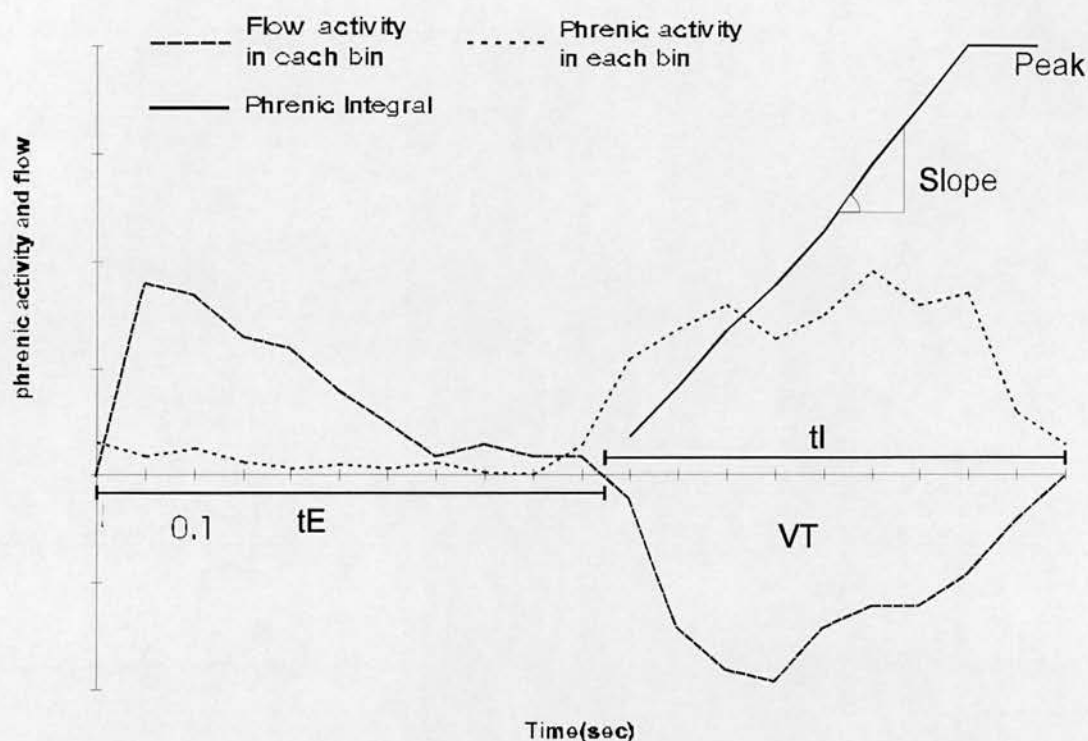


Fig. 2.13 Variables calculated by the script written for the “spike2” analysis package. See text for details.

The program calculated the expiration time (t_E), inspiration time (t_I), and tidal volume (V_T) from flow integration (Fig. 2.13). Phrenic activity was partitioned into 50ms bins and the activity was measured in arbitrary units by the program. The phrenic activity during inspiration was integrated, and the peak and the slope of this integral was calculated. The last bin of integrated phrenic activity was not used in this calculation.

Each of the stages in the three parts of the experiment were analysed as follows:

Control breathing: 5 consecutive breaths (preSO₂, postSO₂, postvagotomy)

Inflation & deflation steps: 3 pre-inflation/deflation breaths (control); breath 1,2&5 of inflation/deflation (except with +10cmH₂O on inflation when only the first breath was measured as a sustained H.B. reflex was produced).

4&6%CO₂: 5 consecutive breaths before applying CO₂ (control) and 5 consecutive breaths at the end of two minutes of CO₂.

Statistical analysis was performed according to the advice of Prof. G. Murray of the Department of Public Health Sciences, University of Edinburgh. Statistical analysis for the following variables: expiratory time (t_E), inspiratory time (t_I), tidal volume (V_T), minute ventilation (\dot{V}_I), frequency of breathing (f), phrenic gradient (G), and phrenic height (H) was performed as follows:

Control breathing

To examine the effects of SO₂ and bilateral vagotomy within groups (normal and emphysema), Repeated Measures ANOVA on Ranks (Student-Newman-Keuls Test) was used to compare postSO₂ to preSO₂ and postvagotomy to postSO₂.

To check for differences between groups [emphysema (E) vs. normal (N)] two methods were used. First, raw data were compared using the Rank Sum test e.g. preSO₂ (E) vs. preSO₂ (N), postSO₂ (E) vs. postSO₂ (N), and postvagotomy (E) vs. postvagotomy (N). In a second method within groups (emphysema and normal) postSO₂ was calculated as % change from preSO₂ and postvagotomy was calculated as percentage change from postSO₂ as follows:

$$\% \text{ change in } t_E = t_{E \text{ postSO}_2 \text{ or Postvagotomy}} - t_{E \text{ preSO}_2} / t_{E \text{ preSO}_2} \cdot 100$$

$$\% \text{ change in } t_E = t_{E \text{ Postvagotomy}} - t_{E \text{ postSO}_2} / t_{E \text{ postSO}_2} \cdot 100$$

Then % change of E was then compared to the corresponding % change of N using the Rank Sum Test e.g. $\Delta\%$ postSO₂ (E) vs. $\Delta\%$ postSO₂ (N), and $\Delta\%$ postvagotomy (E) vs. $\Delta\%$ postvagotomy (N).

Inflation and deflation steps

To examine the effects of inflation and deflation steps on the 1st, 2nd and 5th breaths within groups (emphysema and normal), and before SO₂ (preSO₂) and after

SO₂ (postSO₂), Repeated Measures ANOVA on Ranks (Student-Newman-Keuls Test) was used to compare the 1st, 2nd & 5th breaths to control.

To check for differences between groups (emphysema, E vs. normal, N) preSO₂ and postSO₂, 1st, 2nd & 5th breaths were calculated as % change from control breath as follows:

$$tE_{1st, 2nd \text{ or } 5th} - tE_{control} / tE_{control} \cdot 100$$

Then the % change of emphysema (E) was compared to corresponding % change of normal (N) using the Rank Sum Test e.g. $\Delta\%1^{st}(\text{preSO}_2, E)$ vs. $\Delta\%1^{st}(\text{preSO}_2, N)$, $\Delta\%2^{nd}(\text{preSO}_2, E)$ vs. $\Delta\%2^{nd}(\text{preSO}_2, N)$, and $\Delta\%5^{th}(\text{preSO}_2, E)$ vs. $\Delta\%5^{th}(\text{preSO}_2, N)$. The same procedure was performed for postSO₂: $\Delta\%1^{st}(\text{postSO}_2, E)$ vs. $\Delta\%1^{st}(\text{postSO}_2, N)$, $\Delta\%2^{nd}(\text{postSO}_2, E)$ vs. $\Delta\%2^{nd}(\text{postSO}_2, N)$, and $\Delta\%5^{th}(\text{postSO}_2, E)$ vs. $\Delta\%5^{th}(\text{postSO}_2, N)$.

Accelerated breathing with 4 & 6% CO₂

To examine for the effect of 4 & 6% CO₂ within groups (emphysema and normal) under the condition of preSO₂, postSO₂ & postvagotomy, Repeated Measures ANOVA on Ranks (Student-Newman-Keuls Test) was used to compare the result of 4 & 6% CO₂ to control.

To examine for differences between groups (emphysema, E vs. normal, N) preSO₂, postSO₂ & postvagotomy in response to 4 & 6% CO₂ each response was calculated as % change from control breath as follows:

$$tE_{4 \& 6\% CO_2} - tE_{control} / tE_{control} \cdot 100$$

Then the % change of emphysema (E) was compared to the corresponding % change of normal (N) using Rank Sum Test e.g. $\Delta\%4\%CO_2(\text{preSO}_2, E)$ vs. $\Delta\%4\%CO_2(\text{preSO}_2, N)$, and $\Delta\%6\%CO_2(\text{preSO}_2, E)$ vs. $\Delta\%6\%CO_2(\text{preSO}_2, N)$. The same procedure was performed for postSO₂: $\Delta\%4\%CO_2(\text{postSO}_2, E)$ vs. $\Delta\%4\%CO_2(\text{postSO}_2, N)$, and $\Delta\%6\%CO_2(\text{postSO}_2, E)$ vs. $\Delta\%6\%CO_2(\text{postSO}_2, N)$. The same procedure was also performed for postvagotomy: $\Delta\%4\%CO_2(\text{postvagotomy}, E)$ vs. $\Delta\%4\%CO_2(\text{postvagotomy}, N)$, and $\Delta\%6\%CO_2(\text{postvagotomy}, E)$ vs. $\Delta\%6\%CO_2(\text{postvagotomy}, N)$.

Chapter 3

3. RESULTS

3.1 CHARACTERISTICS OF RABBIT EMPHYSEMA

3.1.1 COMPLIANCE, MEAN LINEAR INTERCEPT, FRESH LUNG WEIGHT AND LUNG VOLUME

Lung compliance was measured in 20 rats: 10 normal and 10 emphysematous (papain induced), and in 67 Dutch rabbits: 25 normal and 42 emphysematous (in 7 emphysema was induced with 240IU/kg elastase, and in 35 emphysema was induced with 400IU/kg elastase). Dutch rabbits' fresh lung weight and fixed lung volume were also measured (Table 3.1).

Table 3.1 Lung compliance of normal and emphysematous animals measured from the volume-pressure deflation curve (def), and that measured from the inflation volume-pressure curve (inf). Data are mean \pm s.d., comparisons made by Mann-Whitney rank sum test. * $p\leq 0.05$, ** $p\leq 0.01$, normal vs. emphysematous animals

	compliance(def) (ml/cmH ₂ O)	compliance(inf) (ml/cmH ₂ O)	fresh lung weight(g)	fixed lung volume (ml)
normal rabbit	8.24 \pm 0.98	4.5 \pm 1.5	12.41 \pm 2.4	44.43 \pm 5.28
emphysematous rabbit (240IU elastase)	9.3 \pm 0.32 *	6.4 \pm 1.2 **	12.6 \pm 3.84	46.84 \pm 6
emphysematous rabbit (400IU elastase)	9.2 \pm 0.62 *	6.4 \pm 2.4 *	14.24 \pm 3.8	49.87 \pm 5.86
normal rat	0.44 \pm 0.01	0.24 \pm 0.02		
emphysematous rat (papain)	0.65 \pm 0.02 **	0.49 \pm 0.06 *		

It can be seen (Table 3.1) that static compliance was increased by pulmonary emphysema induced by 240IU and 400IU elastase and by papain. However neither fresh lung weight nor fixed lung volume were changed.

Mean linear intercept (Lm) was measured in 20 rats: 10 normal and 10 emphysematous and 3 blocks were obtained from the left lobe of each animal. Lm of rabbits was obtained by sampling from all lobes (except the accessory lobe) and was measured in 14 Dutch rabbits: seven normal, seven emphysematous, where emphysema was induced with 240IU/kg elastase, and in 60 Dutch rabbit: 25 normal and 35 emphysematous, where emphysema was induced with 400IU/kg elastase. Emphysematous and normal lungs were processed identically (Table 3.2).

Table 3.2 Mean linear intercept (Lm) of normal and emphysematous rats and Dutch rabbits. Data are mean \pm s.d., comparison made by Mann-Whitney rank sum test, * $p\leq 0.05$, normal vs. emphysematous

	Lm (μ m)
normal rat	80.5 \pm 2.9
emphysematous rat (papain)	108.4 \pm 3.2 *
normal Dutch rabbit	75.87 \pm 9.61
emphysematous D. rabbit (240IU)	103.22 \pm 6.77 *
normal Dutch rabbit	79.71 \pm 8.42
emphysematous D. rabbit (400IU)	99.62 \pm 2.32 *

It is clearly seen (Table 3.2) that mean linear intercept was significantly increased by pulmonary emphysema induced by 240IU and 400IU elastase and by papain.

Since all lobes of rabbits' lungs were sampled the degree of each lobe involvement was evaluated by calculating the Lm of emphysematous as a percentage change from normal. Comparison was also made between emphysematous lobes and corresponding normal lobes (Table 3.3).

Table 3.3 Mean linear intercept (Lm) of rabbits' lungs as raw data and as a percentage change from normal, RU: right upper lobe, RM: right middle lobe, RL: right lower lobe, LU: left upper lobe, LL: left lower lobe. Data are mean \pm s.d., comparison made by Mann-Whitney rank sum test. * $p\leq 0.05$, normal vs. emphysematous lobes

	Lm (μ m)				
	RU	RM	RL	LU	LL
normal rabbit	72.7 \pm 2.3	82.9 \pm 2.6	78.8 \pm 2.3	73.3 \pm 2.1	71.7 \pm 1.7
emphysematous (240IU)	102.1 \pm 3.7*	105.3 \pm 3.7*	103.4 \pm 3.5*	105.6 \pm 3 *	99.7 \pm 3*
$\Delta\%$	40	27	31	44	39
normal rabbit	79.7 \pm 2	84.7 \pm 2.6	82.9 \pm 2.5	81 \pm 3.2	70.3 \pm 1.9
emphysematous (400IU)	93.9 \pm 2.8 *	113.9 \pm 7*	97.8 \pm 4*	99.5 \pm 3.6*	93 \pm 3.6*
$\Delta\%$	18	35	18	23	32

It can be seen (Table 3.3) that all the lobes in the rabbits' lungs have been affected by emphysema as the Lm is significantly increased in all cases.

Alveolar space enlargement due to emphysema induced by 400IU elastase is clearly seen in photomicrographs of alveoli at lower (Fig. 3.1 & 3.2) and at higher magnifications (Fig. 3.3 & 3.4).



Fig. 3.1 Photomicrograph of alveoli from normal rabbits. Original magnification x100.

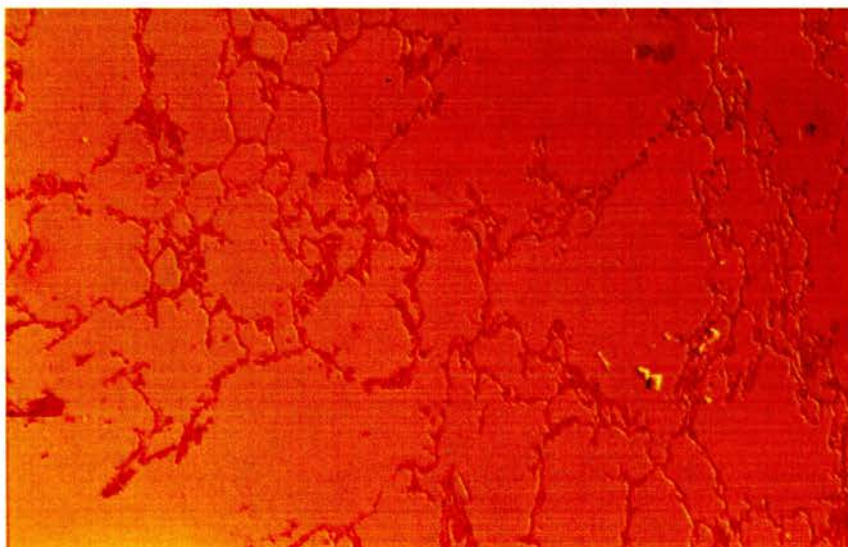


Fig. 3.2 Photomicrograph of alveoli from emphysematous rabbits treated with 400IU/kg elastase. Original magnification x100.

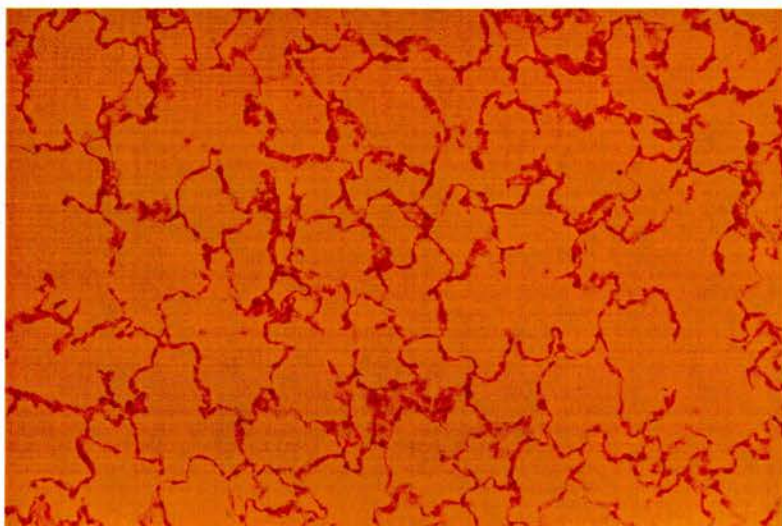


Fig. 3.3 Photomicrograph of alveoli from normal rabbits. Original magnification x200.

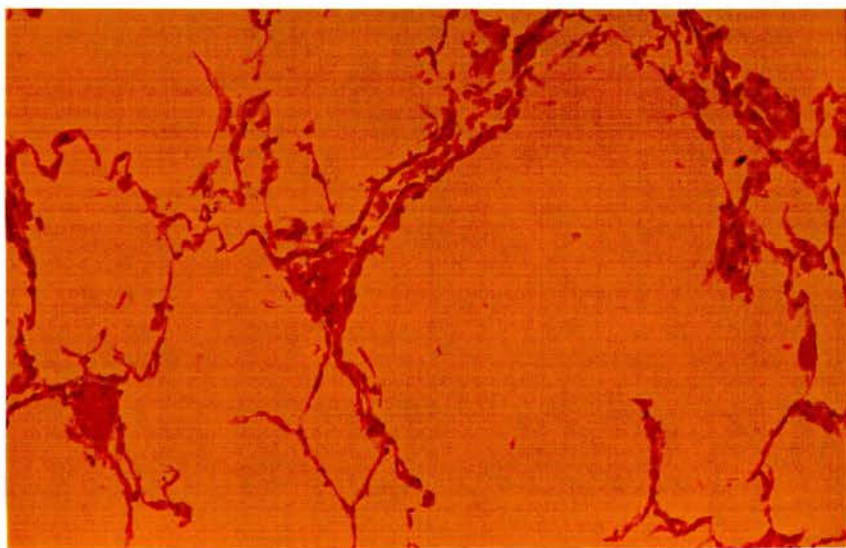


Fig. 3.4 Photomicrograph of alveoli from emphysematous rabbits treated with 400IU/kg elastase. Original magnification x200.

3.1.2 BLOOD GASES, END-TIDAL CO₂ AND BLOOD PRESSURE

During acute experiments, end-tidal CO₂ and arterial blood pressure were measured in 25 normal Dutch rabbits and 35 emphysematous rabbits (emphysema induced with 400IU/kg elastase), whilst arterial blood gases (Pao₂ & Paco₂) were measured in seven normal and seven emphysematous Dutch rabbits (Table 3.4, Fig. 3.5, Table 3.5, 3.6).

Table 3.4 Arterial blood gases of normal & emphysematous Dutch rabbit measured before and after the animals breathed 100% O₂ for two min. Pao₂: arterial oxygen tension, Paco₂: carbon dioxide arterial tension. Data are mean±s.d.

	Before 100% O ₂		After 100% O ₂	
	Pao ₂ (mmHg)	Paco ₂ (mmHg)	Pao ₂ (mmHg)	Paco ₂ (mmHg)
Normal D. rabbit	69.43±18.7	37.34±2.46	462±62	39±3.5
Emphysematous D. rabbit	65.8±17	37.2±3.6	338±64	38.5±4.9

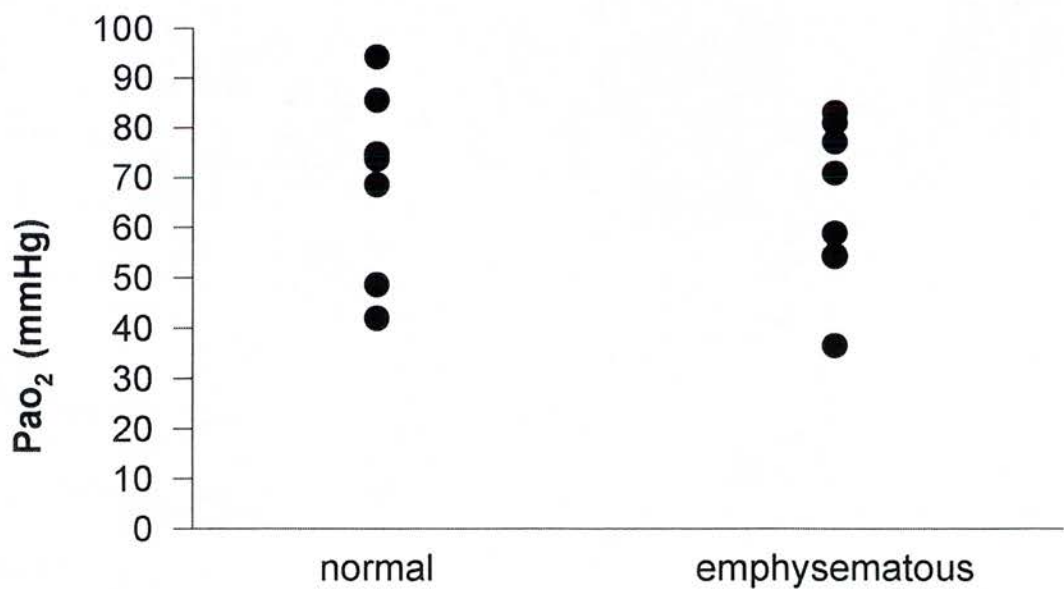


Fig. 3.5 Scatter diagram showing the arterial oxygen tension (Pao₂) in seven normal and seven emphysematous rabbits in the resting state (before 100% O₂ administered).

Results showed that some emphysematous rabbits were hypoxic and so too were some normal rabbits. $Paco_2$ was normal in both normal and emphysematous rabbits (Table 3.4, Fig. 3.5). Administration of 100% oxygen, increased the Pao_2 in normal and emphysematous rabbits (normal > emphysematous).

End-tidal $CO_2\%$ was measured at the end of 10 minutes of control breathing and at the end of 2 minutes of accelerated breathing with 4 and 6% CO_2 . Pre SO_2 is the first part of the experiment where pulmonary receptors were intact, post SO_2 is the second part of the experiment where pulmonary stretch receptors were blocked with sulphur dioxide and postvagotomy is the third part of the experiment where both vagi had been sectioned (Table 3.5).

Table 3.5 End-tidal CO_2 of normal & emphysematous Dutch rabbit during control and accelerated breathing with 4&6% CO_2 . Data are mean \pm s.d.

		end-tidal $CO_2\%$ of normal rabbit	end-tidal $CO_2\%$ of emphysematous rabbit
pre SO_2	Control	3.35 ± 0.66	3.8 ± 0.55
	4% CO_2	5.7 ± 0.15	5.84 ± 0.2
	6% CO_2	7.16 ± 0.2	7.16 ± 0.2
post SO_2	control	3.56 ± 1	3.56 ± 0.9
	4% CO_2	6.13 ± 0.8	5.89 ± 0.6
	6% CO_2	7.6 ± 0.5	7.29 ± 0.45
postvagotomy	control	4.12 ± 0.46	4.1 ± 0.57
	4% CO_2	6.55 ± 0.38	6.23 ± 0.38
	6% CO_2	7.92 ± 0.42	7.53 ± 0.32

This table (Table 3.5) shows that there seems to be no significant differences between normal and emphysematous rabbits.

Table 3.5 shows that in both normal and emphysematous rabbits end-tidal CO₂ increased with 4 & 6% CO₂ being breathed, and both sets of animals have a normal end-tidal CO₂.

During the experiments the blood pressure of normal and emphysematous rabbits Dutch rabbits was recorded at the commencement of the experiment. Values obtained are shown in Table 3.6. Both sets of animals have normal and similar systolic, diastolic and mean blood pressure

Table 3.6 Blood pressure of normal and emphysematous Dutch rabbits, SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean blood pressure determined as DBP plus one-third of the pulse pressure. Data are mean \pm s.d.

	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)
Normal D. rabbit	105 \pm 8.9	72 \pm 7.2	83 \pm 8.2
Emphysematous D. rabbit	109 \pm 10.8	79 \pm 8.5	89 \pm 9.3

3.2 CONSCIOUS PATTERN OF BREATHING

3.2.1 CONSCIOUS PATTERN AS COMPONENT OF VARIANCE AND SAMPLE SIZE

The conscious pattern of breathing of four Dutch rabbits, four New Zealand white rabbits and four rats was measured by the barometric method (as described in the method chapter, page 72). The pattern of breathing was measured on four consecutive days, for 40-100 breath/day/run. Breaths were analysed in terms of their inspiration time (t_i), expiration time (t_e) and tidal volume (V_T) (Table 3.7).

Table 3.7 Pattern of breathing of rats and Dutch (D) & New Zealand white (NZ) rabbits. t_i : inspiration time, t_e : expiration time, V_T : tidal volume, f : breath/min. Data are mean \pm s.d.

	Breaths no.	t_i (sec)	t_e (sec)	V_T	f
D. rabbit	660	0.53 \pm 0.13	0.66 \pm 0.37	13.25 \pm 3.2(ml)	50.4
NZ. rabbit	1535	0.3 \pm 0.1	0.35 \pm 0.11	8.91 \pm 4.2(ml)	92.31
Rat	1600	0.22 \pm 0.04	0.31 \pm 0.09	2.01 \pm 0.59(ml)	113.2

The breathing pattern was compared in conscious emphysematous and normal animals. To estimate the minimum number of animals required to give a statistically valid result variability of pattern of breathing was calculated as component of variance (Dallak, Luan, Brown & Pirie, 1995) by the method of residual maximum likelihood (REML) using the program PROC MIXED IN SAS (SAS Institute Inc., SAS circle, Box 8000 Cary, NC 27512-8000). Variance components calculated were: between days (σ_d^2), between subjects (σ_s^2), between days within subjects (σ_{sd}^2) and within subjects and days (σ_r^2) (Tables 3.8, 3.9, 3.10, 3.11).

Table 3.8 Components of variance of Dutch rabbits pattern of breathing as raw data and as a percentage (%) of total variance.

D. RABBIT						
	ti	%	te	%	V _T	%
between days (σ_d^2)	0	0.00	0.001	0.72	0	0.00
between subjects (σ_s^2)	0.0085	51.20	0.109	78.99	0.315	69.69
between days within subjects (σ_{sd}^2)	0.0046	27.71	0.019	13.77	0.051	11.28
within subjects and days (σ_r^2)	0.0035	21.08	0.009	6.52	0.086	19.03
total variance	0.0166	100	0.138	100	0.452	100

Table 3.9 Components of variance of rats pattern of breathing as raw data and as a percentage (%) of total variance.

RAT						
	ti	%	te	%	V _T	%
between days (σ_d^2)	0	0.00	0.0009	7.50	0.0011	7.19
between subjects (σ_s^2)	0.00012	7.19	0.0002	1.67	0.0008	5.23
between days within subjects (σ_{sd}^2)	0.00041	24.55	0.0037	30.83	0.004	26.14
within subjects and days (σ_r^2)	0.00114	68.26	0.0072	60.00	0.0094	61.44
total variance	0.00167	100	0.012	100	0.0153	100

Table 3.10 Components of variance of New Zealand white rabbits pattern of breathing as raw data and as a percentage (%) of total variance. Data were calculated from the four days of measurement.

New Z. Rabbit	t _I	%	t _E	%	V _T	%
between days (σ_d^2)	0.0025	20.33	0.0024	17.14	0.169	21.67
between subjects (σ_s^2)	0.0023	18.70	0.0023	16.43	0.09	11.54
between days within subjects (σ_{sd}^2)	0.0044	35.77	0.0054	38.57	0.394	50.51
within subjects and days (σ_r^2)	0.0031	25.20	0.0039	27.86	0.127	16.28
total variance	0.0123	100	0.014	100	0.78	100

Table 3.11 Components of variance of New Zealand white rabbits pattern of breathing as raw data and as a percentage (%) of total variance Data were calculated from the last two days of the four days of measurement.

New Zealand Rabbit	t _I	%	t _E	%	V _T	%
between days (σ_d^2)	0.0003	4.84	0.0008	8.70	0.002	0.96
between subjects (σ_s^2)	0.0032	51.61	0.0042	45.65	0.007	3.37
between days within subjects (σ_{sd}^2)	0.0004	6.45	0.0008	8.70	0.11	52.88
within subjects and days (σ_r^2)	0.0023	37.10	0.0034	36.96	0.089	42.79
total variance	0.0062	100	0.0092	100	0.208	100

To explain the implication of the component of variance, Dutch rabbit t_I components of variance may be used as an example. Total variance (0.0166) (Table 3.8) is the estimated variance of a single t_I from a randomly chosen rabbit, and

analysis shows that of this total variance, 51% is estimated to be attributable to systematic difference between rabbits (σ_s^2), 28% attributable to interaction of days and rabbits (σ_{sd}^2) (an interaction means that variability on different days is different from rabbit to rabbit) and 21% is attributable to residual variation (σ_r^2), which reflects variation in breaths measured on the same day.

The sample size of animal required to detect a significant change in the mean with 80% power was calculated by the following equation, which was provided by Dr. H. Brown of Department of Public Health Sciences, Medical School, Edinburgh University

$$d = (Z_{\alpha/2} + Z_{1-\beta}) \times \text{SQRT} (2 (\sigma_d^2 / n_d + \sigma_{sd}^2 / (n_s \times n_d) + \sigma_r^2 / (n_s \times n_d \times n_r)))$$

where d: mean difference, $Z_{\alpha/2}$ and $Z_{1-\beta}$ are percentage points on the normal distribution corresponding to the significance level, α ($Z_{\alpha/2}=1.96$ for $\alpha=0.05$), and the power, β ($Z_{1-\beta}=0.84$ for 80% power), SQRT: square root, n_d : number of days, n_s : number of subjects, n_r : number of observations (i.e. breaths), σ_d^2 : between days variance component, σ_{sd}^2 : between days within subjects variance component, σ_r^2 : within subjects and days variance component (Table 3.12, 3.13).

Table 3.12 Number (no.) of Dutch rabbits and rats required to detect a certain % difference (5%, 10% & 20%) from the mean of breathing pattern parameters (t_i , t_E , V_T).

	% Difference	D. Rabbit no.	Rat no.
t_i	5%	38	14
	10%	10	4
	20%	3	1
t_E	5%	73	31
	10%	19	8
	20%	10	2
V_T	5%	10	22
	10%	3	6
	20%	1	2

Table 3.13 Number of New Zealand white rabbits required to detect a certain % difference from the mean of breathing pattern (ti, tE, VT). Sample size was obtained from the last two days of the four days of measurement.

NZ. Rabbits no.	% difference		
	ti	tE	VT
10	15%	19%	14%
15	14%	19%	12%
20	14%	19%	10%

The variability of breathing pattern of New Zealand white rabbits measured for the first time for four consecutive days was initially high but declined and therefore data from the last 2 days were used to calculate sample size, since rabbits became calmer and this caveat could be built into the experimental design. This in itself probably shows behavioural differences between Dutch and New Zealand white rabbits since the same protocol was used with both types (see method section). Differences between the two strains of rabbits is also shown by the sample size required, where a larger number of New Zealand white rabbits is required to detect a similar % change in the mean. Therefore Dutch rabbits were used as the subjects of this study.

Differences between species in component of variance were examined by calculating each variance component as a percentage of the total variance for that particular species (Tables 3.8, 3.9, 3.10, 3.11 and Fig. 3.6 & 3.7).

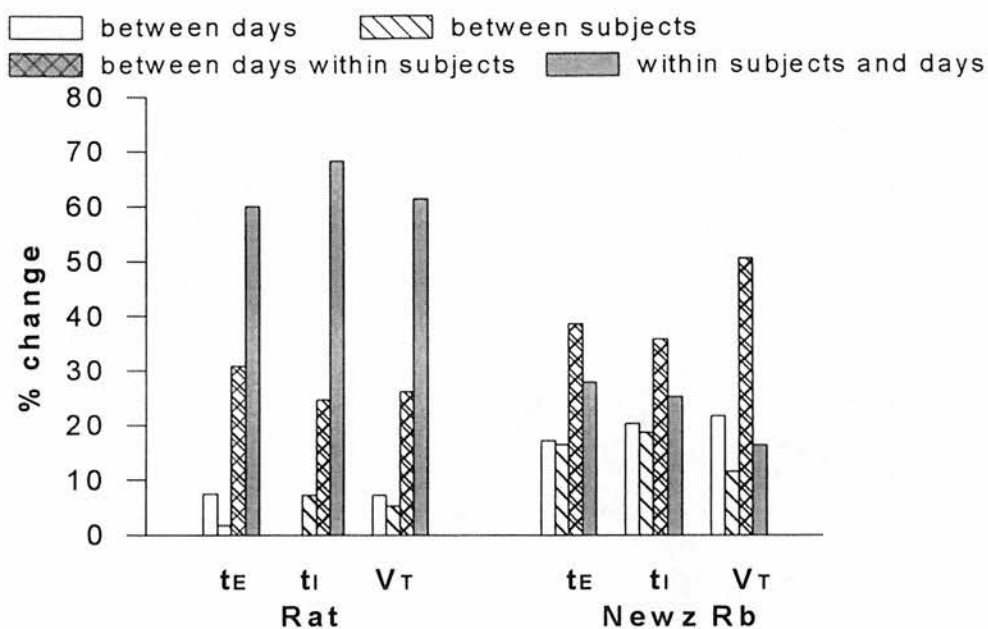


Fig. 3.6 Rats and New Zealand white rabbit (New z Rb) component of variance as percentage of total variance.

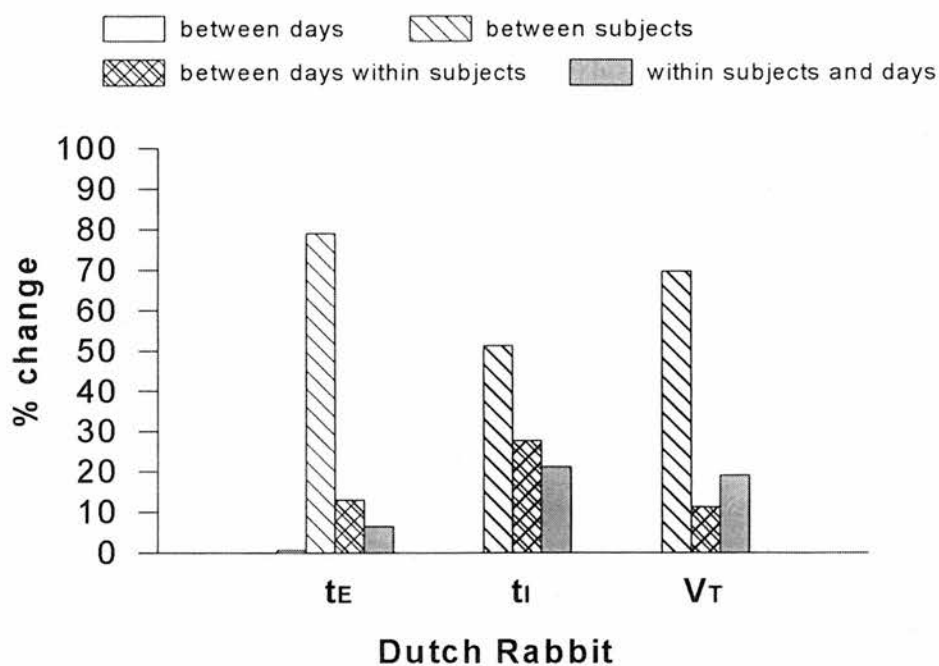


Fig. 3.7 Dutch rabbit component of variance as percentage of total variance.

The results shown in Figures 3.6 & 3.7 show that the source of highest variability is the same for the three parameters (t_i , t_e , V_T) in each species. The source of highest variability in Dutch rabbits is between subjects while in rats it is the within subjects and days and in New Zealand white rabbits it is between days within subjects.

3.2.2 CONSCIOUS PATTERN OF BREATHING BEFORE AND AFTER EMPHYSEMA

To examine the effect of emphysema on the conscious pattern of breathing, emphysema was induced in seven Dutch rabbits by giving type IV porcine pancreatic elastase (240 units/kg body weight in 1ml sterile 0.9% saline) by insufflation and in ten rats using papain (120 mg/kg in 0.25ml 0.9% saline) (as described in the method chapter).

The pattern of breathing was measured by the barometric method on four consecutive days, 20 breaths each day before and after emphysema had been induced. Rabbits were left for four weeks for emphysema to develop, whilst rats were left for two weeks. The mean of the four days measurement was calculated and comparison between means (before and after emphysema) was made by Wilcoxon signed-rank test; $p \leq 0.05$ was taken as significant.

Variables measured were inspiratory time (t_i) and expiratory time (t_e) in sec; tidal volume (V_T) in ml; breathing frequency (f) in breaths/min; minute ventilation (\dot{V}_I) in l/min;

Rabbits

In Dutch rabbits, although there was a mean decrease in t_e and t_i , and thus an increase in f , these changes did not reach significance because of the variability in the pattern of breathing (Table 3.14). For example in the case of t_i four rabbits showed a decrease whereas three showed an increase in inspiratory time. Similarly for V_T , four rabbits showed a decrease whereas three rabbits showed an increase in tidal volume.

Rats

For rats emphysema caused no significant change in t_i (before: 0.21 ± 0.02 ; after: 0.22 ± 0.02). However there was a significant increase in t_E (before: 0.31 ± 0.06 ; after: 0.4 ± 0.07); thus there was a significant decrease in f (before: 115.6 ± 4.6 ; after: 97.6 ± 4). V_T increased significantly (before: 2.02 ± 0.3 ; after: 2.45 ± 0.3). But \dot{V}_I did not show a significant change because although there was an increase in V_T , this was associated with a decrease in f (Table 3.14).

Table 3.14 Pattern of breathing (t_i , t_E , V_T) and minute ventilation (\dot{V}_I) of Dutch rabbits and rats before and after emphysema developed. Data are mean \pm s.d, ** $p\leq0.01$, *** $p\leq0.001$, before emphysema vs. after emphysema.

		D. rabbit	rat
t_i (sec)	before	0.54 ± 0.24	0.21 ± 0.02
	after	0.42 ± 0.11	0.22 ± 0.02
t_E (sec)	before	0.67 ± 0.2	0.31 ± 0.06
	after	0.5 ± 0.13	0.4 ± 0.07 **
f (breath/min)	before	54 ± 5.4	115.6 ± 4.6
	after	66.3 ± 5.5	97.6 ± 4 ***
V_T (ml)	before	13.3 ± 2.9	2.02 ± 0.3
	after	12.6 ± 3.1	2.45 ± 0.3 **
\dot{V}_I (l/min)	before	0.7 ± 0.07	0.23 ± 0.01
	after	0.83 ± 0.05	0.24 ± 0.01
weight (g)	before	1993 ± 193	307 ± 33
	after	2287 ± 206 **	376 ± 47 ***

3.3 ACUTE EXPERIMENTS

The variables measured were: inspiratory time (t_i), expiratory time (t_E) in sec; tidal volume (V_T) in ml; breathing frequency (f) in breaths/min; minute ventilation (\dot{V}_I) in l/min; phrenic slope (G) and height (H) in computer arbitrary units

For comparison to be made between preSO₂, postSO₂ and postvagotomy for the given variable within a group, the raw data was analysed using Repeated Measures ANOVA on Ranks (Student-Newman-Keuls Test).

Each experiment consisted of 3 parts. Part 1 is preSO₂, where all pulmonary receptors were intact. Part 2 is postSO₂, where SARs were blocked with SO₂. Part 3 is postvagotomy, where all pulmonary receptors were removed by bilateral vagotomy. In parts 1 & 2 there were three stages that have been analysed: control breathing, breathing against positive and negative pressure, and breathing accelerated with 4 & 6% CO₂. In part 3 there were only two stages: control breathing and breathing accelerated with 4 & 6% CO₂. Detailed description of the protocol is summarised in Table 2.1 (page 103).

3.3.1 EFFECTS OF SO₂ AND BILATERAL VAGOTOMY ON PATTERN OF BREATHING, VENTILATION & PHRENIC ACTIVITY

Normal rabbits

The effects of removal of slowly adapting pulmonary stretch receptors (SARs) activity by SO₂ are shown in Table 3.15 & Fig. 3.8. It can be seen that there was an increase in t_E (0.49 ± 0.02 vs. 0.71 ± 0.03) and t_i (0.39 ± 0.01 vs. 0.59 ± 0.01); therefore f (69.3 ± 1.9 vs. 46.8 ± 0.9) was reduced. SO₂ increased V_T (11.3 ± 0.2 vs. 13.3 ± 0.4) but because of the reduction in f , \dot{V}_I (0.78 ± 0.03 vs. 0.62 ± 0.03) was reduced. Phrenic slope (G) (34.6 ± 1.8 vs. 24.1 ± 1.3) was reduced, while phrenic H (12 ± 0.75 vs. 11.7 ± 0.49) was unchanged.

Bilateral vagotomy (Table 3.15 & Fig. 3.8) further increased t_E (0.71 ± 0.03 vs. 0.95 ± 0.02) and t_i (0.59 ± 0.01 vs. 0.66 ± 0.02), therefore an additional reduction in f (46.8 ± 0.9 vs. 37.2 ± 0.4) was produced. V_T (13.3 ± 0.4 vs. 16 ± 0.7) was further increased, however \dot{V}_I (0.62 ± 0.03 vs. 0.6 ± 0.03) was unchanged. Phrenic G

(24.1 ± 1.3 vs. 23.1 ± 1.8) was not significantly altered by vagotomy, however phrenic H (11.7 ± 0.49 vs. 12.5 ± 0.76) was increased significantly.

Emphysematous rabbits

In emphysematous rabbits, the removal of the SARs (Table 3.15 & Fig. 3.8) by SO_2 also caused an increase in t_i (0.4 ± 0.01 vs. 0.54 ± 0.02), but in contrast to normal rabbits where t_E was markedly increased, there was no significant change in t_E (0.57 ± 0.02 vs. 0.55 ± 0.02). Therefore f (62.9 ± 1.5 vs. 57.2 ± 1.7) was slightly but significantly decreased. V_T (14.7 ± 0.53 vs. 17.7 ± 1) was increased, and so too was \dot{V}_I (0.91 ± 0.02 vs. 0.96 ± 0.03). Phrenic G (49.9 ± 4.1 vs. 54.5 ± 5.3) and H (17.4 ± 1.5 vs. 25.3 ± 2.4) were both increased.

Bilateral vagotomy (Table 3.15 & Fig. 3.8) caused a further increase in t_E (0.55 ± 0.02 vs. 0.8 ± 0.03) and t_i (0.54 ± 0.02 vs. 0.71 ± 0.02). Therefore f (57.2 ± 1.7 vs. 41 ± 1.2) was greatly reduced. V_T (17.7 ± 1 vs. 19.2 ± 0.7) was further increased; however \dot{V}_I (0.96 ± 0.03 vs. 0.76 ± 0.01) was reduced. Phrenic G (54.5 ± 5.3 vs. 41.4 ± 3.9) was reduced however phrenic H (25.3 ± 2.4 vs. 25.7 ± 2.4) was unchanged following vagotomy.

Table 3.15 The effect of SO₂ & vagotomy on the pattern of breathing, ventilation & phrenic activity in normal and emphysematous rabbits. Inspiratory time (ti, sec), expiratory time (te, sec), minute ventilation (\dot{V}_I , l/min), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units), tidal volume (VT, ml), breathing frequency (f, breaths/min), normal Dutch rabbit (N), emphysematous Dutch rabbit (E). Data are mean±SEM, * # p≤0.05. * indicates significance between postSO₂ & postvagotomy vs. preSO₂ . # indicates significance between postSO₂ & postvagotomy.

		PreSO ₂	PostSO ₂	postvagotomy
te	N (n=20)	0.49±0.02	0.71±0.03 *	0.95±0.02 *#
	E (n=35)	0.57±0.02	0.55±0.02	0.8±0.03 *#
ti	N	0.39±0.01	0.59±0.01 *	0.66±0.02 *#
	E	0.4±0.01	0.54±0.02 *	0.71±0.02 *#
\dot{V}_I	N	0.78±0.03	0.62±0.03 *	0.6±0.03 *
	E	0.91±0.02	0.96±0.03 *	0.76±0.01 *#
G	N	34.6±1.8	24.1±1.3 *	23.1±1.8 *
	E	49.9±4.1	54.5±5.3 *	41.4±3.9 *#
H	N	12±0.75	11.7±0.49	12.5±0.76 *#
	E	17.4±1.5	25.3±2.4 *	25.7±2.4 *
VT	N	11.3±0.2	13.3±0.4 *	16±0.7 *#
	E	14.7±0.53	17.7±1 *	19.2±0.7 *#
f	N	69.3±1.9	46.8±0.9 *	37.2±0.4 *#
	E	62.9±1.5	57.2±1.7 *	41±1.2 *#

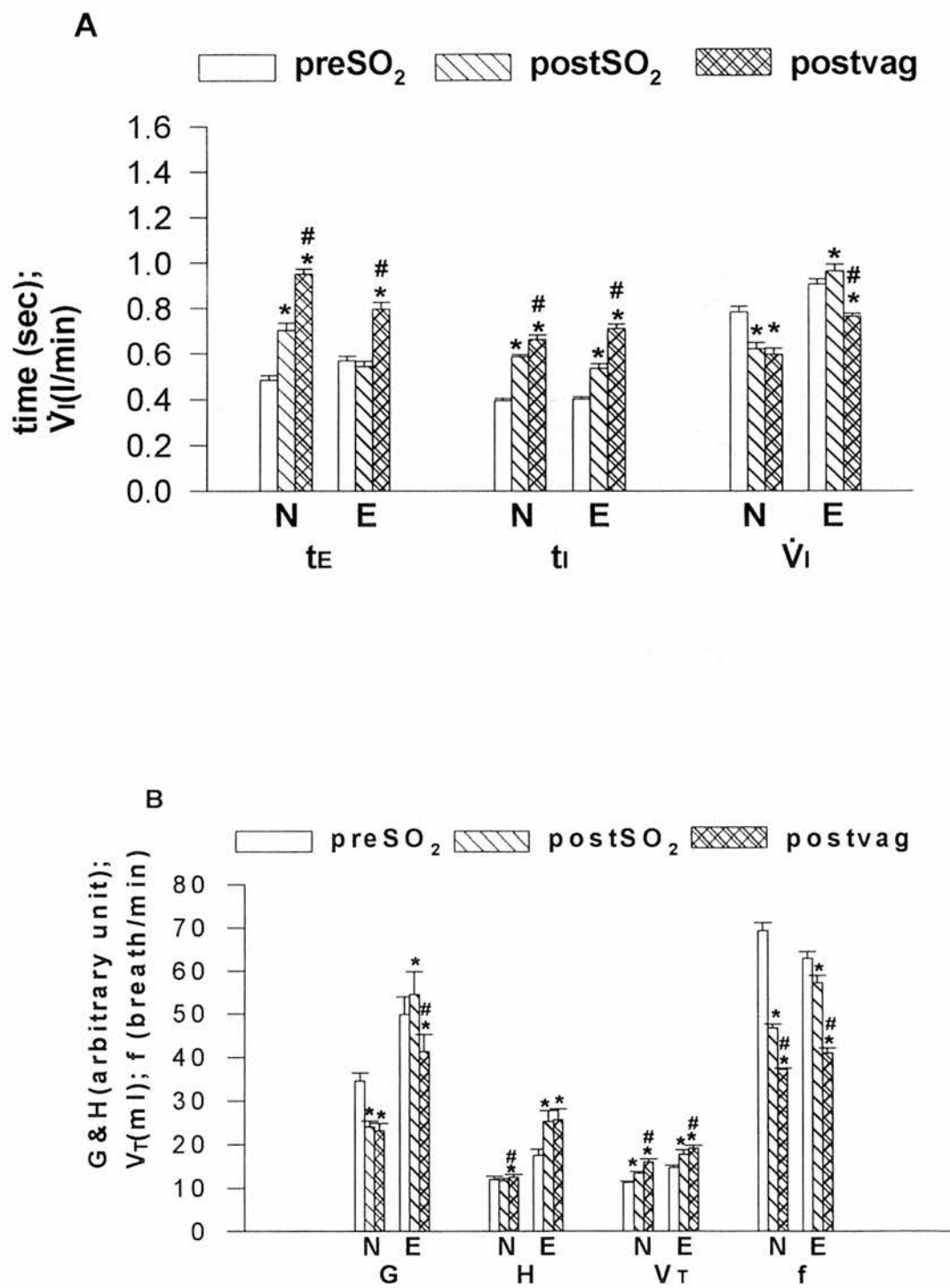


Fig. 3.8 Effect of SO₂ & vagotomy on the pattern of breathing, ventilation & phrenic activity in normal and emphysematous rabbits. Inspiratory time (t_I), expiratory time (t_E), minute ventilation (\dot{V}_I), phrenic gradient (G), phrenic height (H), tidal volume (V_T), breathing frequency (f), normal Dutch rabbit (N), emphysematous Dutch rabbit (E). Data are mean±SEM, * # p≤0.05. * indicates significance between postSO₂ & postvagotomy vs. preSO₂. # indicates significance between postSO₂ & postvagotomy.

3.3.2 COMPARING NORMAL AND EMPHYSEMATOUS RABBITS IN THE CONTROL STATE AND IN THEIR RESPONSE TO SO₂ & BILATERAL VAGOTOMY

Because of the unexplained deaths of five normal rabbits after bilateral vagotomy, the total numbers of normal rabbits is 20 when comparing within normal group e.g. preSO₂ vs. postSO₂ vs. postvagotomy. However when comparing normal to emphysematous rabbits the number of normal rabbits is 25 for preSO₂ & postSO₂ and 20 for postvagotomy.

Comparison of variables between normal and emphysematous animals in the control state was made for the raw data using the Rank Sum Test. However, postSO₂ and postvagotomy parameters were calculated as the % change when examining for differences between normal and emphysematous rabbits in their response to SO₂ and bilateral vagotomy. An example of how the calculations were made is shown for t_E. PostSO₂ as % change from preSO₂:

$$\% \text{ change in } t_E = (t_{E \text{ postSO}_2} - t_{E \text{ preSO}_2}) / t_{E \text{ preSO}_2} \cdot 100$$

The % change of emphysematous rabbits was then compared to corresponding % change of normal animals using the Rank Sum Test.

Postvagotomy as % change from postSO₂:

$$\% \text{ change in } t_E = (t_{E \text{ postvagotomy}} - t_{E \text{ postSO}_2}) / t_{E \text{ postSO}_2} \cdot 100$$

The % change of emphysematous rabbits was compared to corresponding % change of normal animals using the Rank Sum Test.

Control state

When comparing anaesthetised emphysematous (E) to normal (N) animals in the control state (Table 3.16, Fig. 3.9A&B), results showed that t_I was not significantly different (N: 0.38±0.01; E: 0.4±0.01). Emphysematous rabbits had significantly longer t_E (N: 0.49±0.02; E: 0.57±0.02) and thus significantly lower f (N: 69.4±1.5; E: 62.9±1.5). V_T (N: 11.3±0.19; E: 14.7±0.53) was significantly greater in emphysematous rabbits, and so too was V_I (N: 0.78±0.02; E: 0.9±0.02). Phrenic G (N: 38.9±2.29; E: 49.9±4.11) and H (N: 13±0.74; E: 17.4±1.5) were greater in emphysematous rabbits, but the results did not reach significance.

Emphysematous vs. normal rabbits in their response to SO₂ and bilateral vagotomy

SO₂ increased V_T in both normal (15.4±1.9%) and emphysematous rabbits (22.25±5.6%) (Table 3.17, Fig. 3.10A&B), this increase was slightly larger in emphysematous animals although this was not statistically significant. t_I was increased in both normal (46.8±4.5%) and emphysematous (33.6±3.6%) rabbits, but the increase in normal animals was significantly greater. However though t_E was significantly increased in normal rabbits (39.8±3.93%) there was no significant change in emphysematous rabbits (0.7±5.2%) a difference that was very highly statistically significant. In both normal and emphysematous rabbits, f was decreased but the decrease in normal (-28.9±1.6%) was significantly more than in emphysematous animals (-7.2±3.7%). \dot{V}_I in normal rabbits was significantly reduced due to a greater reduction of f (-28.9±1.6%) compared to the increase in V_T (15.4±1.9%). However, \dot{V}_I was increased in emphysematous rabbits because of greater increase in V_T (22.25±5.6%) compared to the decrease in f (-7.2±3.7%). Phrenic G of normal rabbits (-22.2±3.7%) was decreased while that of emphysematous rabbits (10.8±6.6%) was increased. Phrenic H was increased in emphysematous rabbits, but was not changed significantly in normal rabbits, a difference that was statistically significant (N: 9.4±4.7%; E: 47.5±8.6%).

Bilateral vagotomy increased t_E (N: 37.6±4.3%; E: 47.1±2.5%) and t_I (N: 13.5±2.5%; E: 33.3±2.5%) in both normal and emphysematous rabbits (Table 3.17, Fig. 3.10A&B), however this lengthening of t_I and t_E was significantly greater in emphysematous animals, and so too was the reduction in f (N: -19.8±1.9%; E: -28.1±0.9%). Bilateral vagotomy also increased V_T in both normal (20.6±3.4%) and emphysematous rabbits (12.6±2.9%), but the difference in V_T between the two groups did not reach significance (p=0.08). Because of the greater reduction of f in emphysematous animals, the reduction in \dot{V}_I was greater in emphysematous (-19.2±2%) than normal rabbits (-3.8±2.5%). Phrenic G was not changed significantly in normal rabbits (-4.8±4.8%), but it was greatly reduced in emphysematous rabbits (-23.3±2.1%), a difference that was statistically significant. Phrenic H was increased

in normal ($5.9\pm4.2\%$), but was not changed in emphysematous rabbits ($2.3\pm3.8\%$), but this difference was not statistically significant (Table 3.17, Fig. 3.10B).

Table 3.16 Comparison of pattern of breathing, ventilation & phrenic activity between normal (N) & emphysematous (E) Dutch rabbits. Inspiratory time (ti, sec), expiratory time (te, sec), minute ventilation (\dot{V}_I , l/min), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units), tidal volume (VT, ml), breathing frequency (f, breaths/min). Data are mean \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$ & *** $p \leq 0.001$.

		preSO ₂	postSO ₂	postvagotomy
te	N	0.49 \pm 0.02 (n=25)	0.68 \pm 0.02 (n=25)	0.95 \pm 0.02 (n=20)
	E	0.57\pm0.02 * (n=35)	0.55\pm0.02 * (n=35)	0.8\pm0.03 *** (n=35)
ti	N	0.38 \pm 0.01	0.56 \pm 0.02	0.66 \pm 0.02
	E	0.4\pm0.01	0.54\pm0.02	0.71\pm0.02
\dot{V}_I	N	0.78 \pm 0.02	0.64 \pm 0.02	0.6 \pm 0.03
	E	0.9\pm0.02 ***	0.96\pm0.03 ***	0.76\pm0.01 ***
G	N	38.89 \pm 2.29	31.54 \pm 3.2	23.1 \pm 1.8
	E	49.92\pm4.11	54.5\pm5.3 ***	41.4\pm3.9 **
H	N	13 \pm 0.74	14.1 \pm 1.1	12.5 \pm 0.8
	E	17.4\pm1.5	25.3\pm2.4 ***	25.7\pm2.4 **
VT	N	11.31 \pm 0.19	13.1 \pm 0.4	16 \pm 0.7
	E	14.7\pm0.53 ***	17.7\pm1.1 ***	19.2\pm0.7 **
f	N	69.38 \pm 1.5	49.1 \pm 1.2	37.2 \pm 0.4
	E	62.9\pm1.5 ***	57.2\pm1.7 ***	41\pm1.2 ***

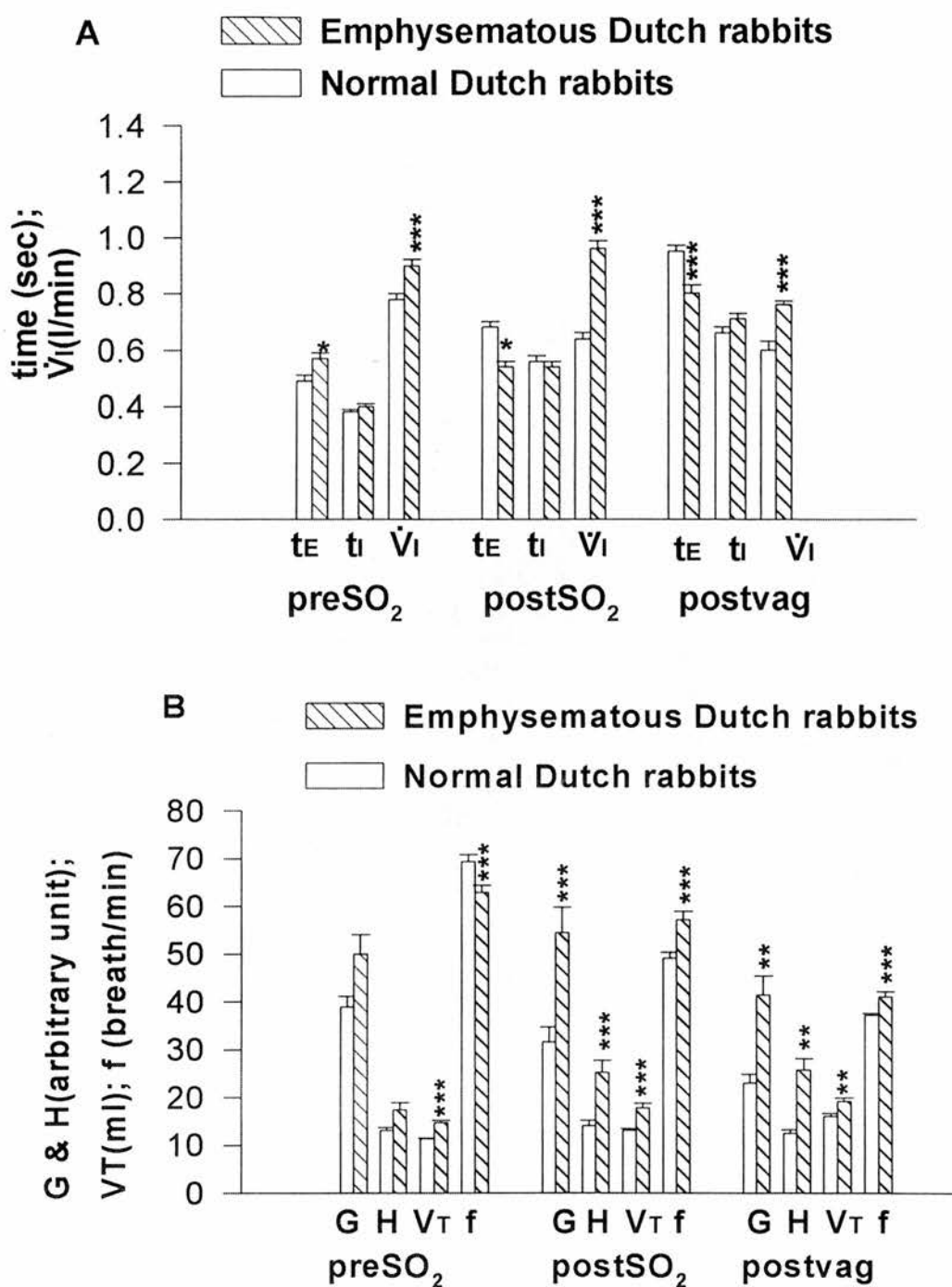


Fig. 3.9 Comparison of pattern of breathing, ventilation & phrenic activity between normal (N) & emphysematous (E) Dutch rabbits. Inspiratory time (tI), expiratory time (tE), minute ventilation (\dot{V}_I), phrenic gradient(G), phrenic height (H), tidal volume (VT), breathing frequency (f). Data are mean \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$ & *** $p \leq 0.001$.

Table 3.17 Comparison of pattern of breathing, ventilation & phrenic activity between normal (N) & emphysematous (E) Dutch rabbits. PostSO₂ was calculated as percentage change from preSO₂ and postvagotomy was calculated as percentage change from postSO₂. Inspiratory time (ti), expiratory time (te), minute ventilation (\dot{V}_I), phrenic gradient (G), phrenic height (H), tidal volume (VT), breathing frequency (f). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

		postSO ₂ % change		Postvagotomy % change
te	N (n=25)	39.8±3.93	N (n=20)	37.6±4.3
	E (n=35)	0.7±5.2 ***	E (n=35)	47.1±2.5 *
ti	N	46.8±4.5	N	13.5±2.5
	E	33.6±3.6 *	E	33.3±2.5 ***
\dot{V}_I	N	-18±2.2	N	-3.8±2.5
	E	7.8±3.3 ***	E	-19.2±2 ***
G	N	-22.2±3.7	N	-4.8±4.8
	E	10.8±6.6 **	E	-23.3±2.1 **
H	N	9.4±4.7	N	5.9±4.2
	E	47.5±8.6 **	E	2.3±3.8
VT	N	15.4±1.9	N	20.6±3.4
	E	22.25±5.6	E	12.6±2.9
f	N	-28.9±1.6	N	-19.8±1.9
	E	-7.2±3.7 ***	E	-28.1±0.9 ***

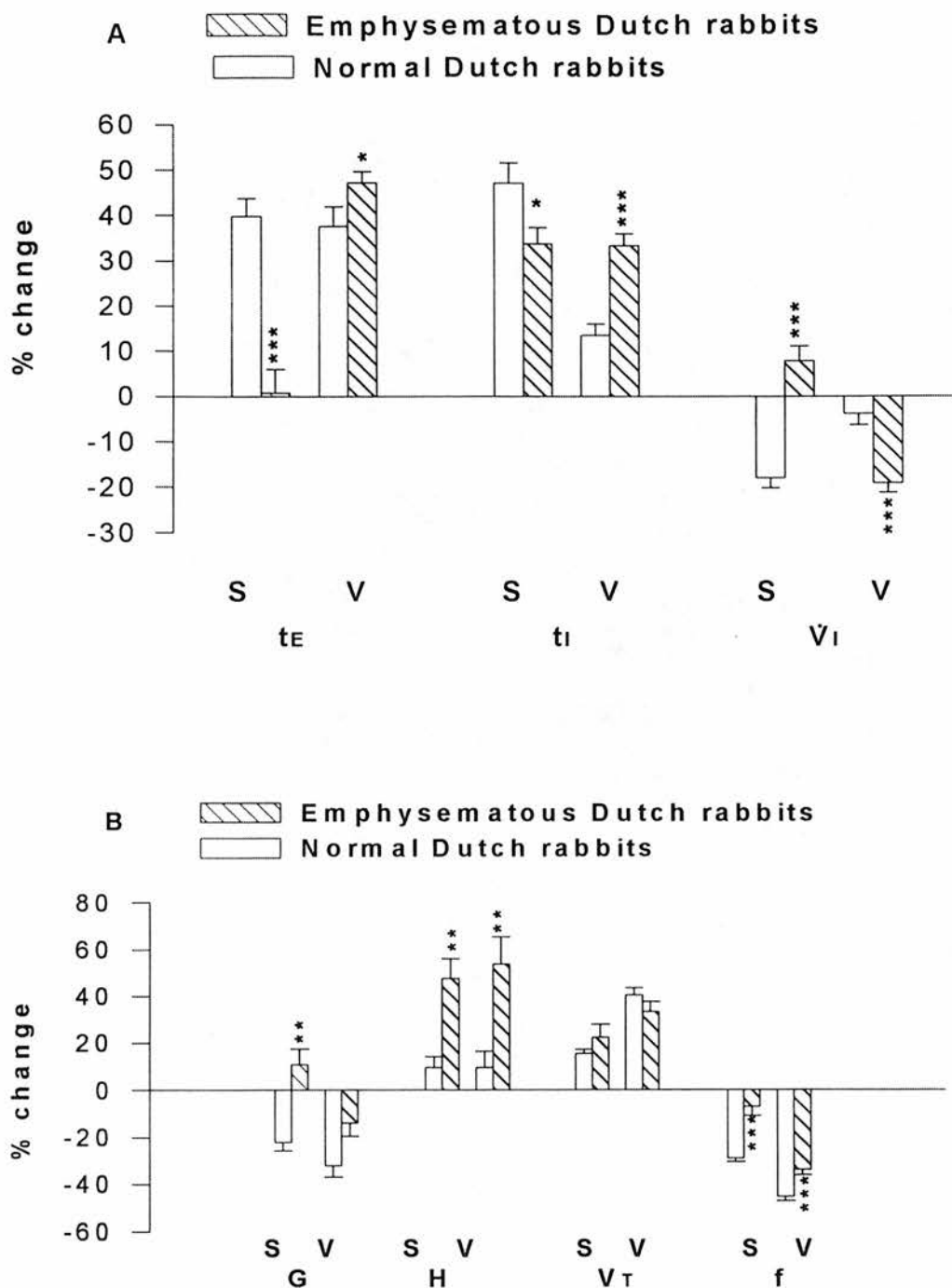


Fig. 3.10 Comparison of pattern of breathing, ventilation & phrenic activity between normal (N) & emphysematous (E) Dutch rabbits. PostSO₂ (S) & postvagotomy (V) were calculated as percentage change from preSO₂. Inspiratory time (ti), expiratory time (tE), minute ventilation (\dot{V}_I), phrenic gradient (G), phrenic height (H), tidal volume (VT), breathing frequency (f). Data are mean \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$ & *** $p \leq 0.001$.

3.3.3 EFFECT OF INFLATION STEPS WITH 5 & 10 cmH₂O

3.3.3.1 EFFECT OF INFLATION STEPS WITH 5 & 10 cmH₂O BEFORE THE ADMINISTRATION OF SO₂ (PRESO₂)

Effect of inflation steps in normal and emphysematous rabbits

Normal and emphysematous rabbits were given 100% O₂ to breathe for 2 minutes before the inflation and deflation steps. These steps were effected by an air pump and an air vacuum respectively, so that during these manoeuvres the animals were breathing air.

To examine the effects of inflation with +5 cm H₂O the mean of 3 breaths before and the 1st, 2nd & 5th breaths during inflation were measured. For inflation with 10 cm H₂O, the control breaths were considered as above, but during the inflation only the 1st breath was measured. Variables analysed were: expiratory time (t_E) and inspiratory time (t_I) in sec, phrenic gradient (G) and phrenic height (H) in computer arbitrary units. Data are presented as the mean \pm SEM and statistical significance was set at $p \leq 0.05$. The changes in t_E are called the Hering-Breuer inflation reflex (HBIR).

To determine the effects of the inflation steps on the above variables within a group, Repeated Measures ANOVA on Ranks (Student-Newman-Keuls Test) was used.

For the inflation step of +5 cm H₂O, it can be seen from Table 3.18A & Fig. 3.11 that in both normal (N) and emphysematous (E) rabbits the t_E of the 1st breath (N: 1.05 \pm 0.09; E: 1.1 \pm 0.06) during inflation was approximately twice that of the control (N: 0.49 \pm 0.01; E: 0.53 \pm 0.02). The t_E's of the 2nd (N: 0.71 \pm 0.06; E: 0.78 \pm 0.04) and 5th breaths (N: 0.66 \pm 0.04; E: 0.71 \pm 0.04) became progressively shorter but they were still significantly longer than the t_E of the control breath. The t_I of the 1st breath was slightly but not significantly increased in both normal and emphysematous rabbits. In both normal and emphysematous rabbits, t_I of the 2nd & 5th breaths were slightly shorter than the control but these small decreases were only significant for the emphysematous rabbits. There were no significant changes in phrenic G & H.

During inflation with 10 cm H₂O (Table 3.18B, Fig. 3.12) there was no change in t_I in normal and emphysematous rabbits, but there was a highly significant increase in t_E for both groups of animals. This was approximately 10 times as long in

normal rabbits (con: 0.45 ± 0.01 ; 1st: 4.53 ± 1.2) and approximately 8 times as long in emphysematous rabbits (con: 0.51 ± 0.02 ; 1st: 3.96 ± 0.6). After the considerable prolongation of TE, there was a significant increase in both phrenic G & H which indicated that the drive to breathe was increased (Table 3.18B, Fig. 3.12).

Table 3.18 Effect of inflation steps with +5 (A) & +10 (B) cmH₂O on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were intact (preSO₂). Inspiratory time (ti, sec), expiratory time (te, sec), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units). Data are mean±SEM. 1st, 2nd & 5th breaths under pressure vs. control breaths (con). With 10 cm H₂O only the 1st breath was measured * p≤0.05, ** p≤0.01 & *** p≤0.001.

A

+5cmH ₂ O	PreSO ₂	con	1st	2nd	5th
N(n=25)	te	0.49±0.01	1.05±0.09 *	0.71±0.06 *	0.66±0.04 *
	ti	0.38±0.01	0.41±0.02	0.36±0.01	0.35±0.01
	G	11.36±0.31	11.32±0.35	11.18±0.25	11.21±0.28
	H	3.79±0.13	4.2±0.39	3.45±0.16	3.41±0.17
E(n=35)	te	0.53±0.02	1.1±0.06 *	0.78±0.04 *	0.71±0.04 *
	ti	0.4±0.01	0.414±0.01	0.398±0.02 *	0.375±0.01 *
	G	11.22±0.39	11.23±0.47	11.43±0.42	11.44±0.38
	H	3.79±0.11	4.15±0.29	3.97±0.29	3.58±0.16

B

+10cmH ₂ O	PreSO ₂	con	1st
N(n=25)	te	0.45±0.01	4.53±1.2 ***
	ti	0.38±0.01	0.41±0.02
	G	11.35±0.29	13.13±0.5 ***
	H	3.7±0.13	4.64±0.32 ***
E(n=35)	te	0.51±0.02	3.96±0.6 ***
	ti	0.39±0.01	0.42±0.02
	G	11.36±0.38	13.44±0.53 ***
	H	3.74±0.13	4.88±0.42 **

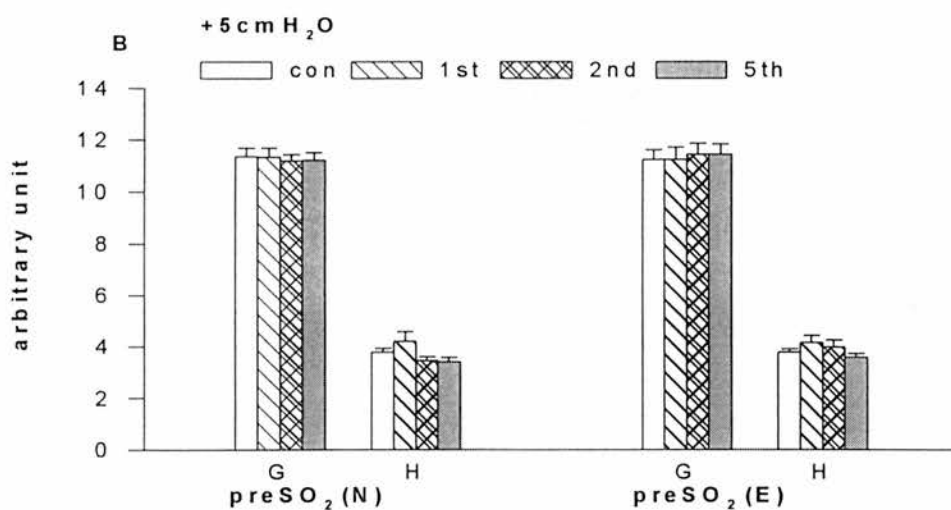
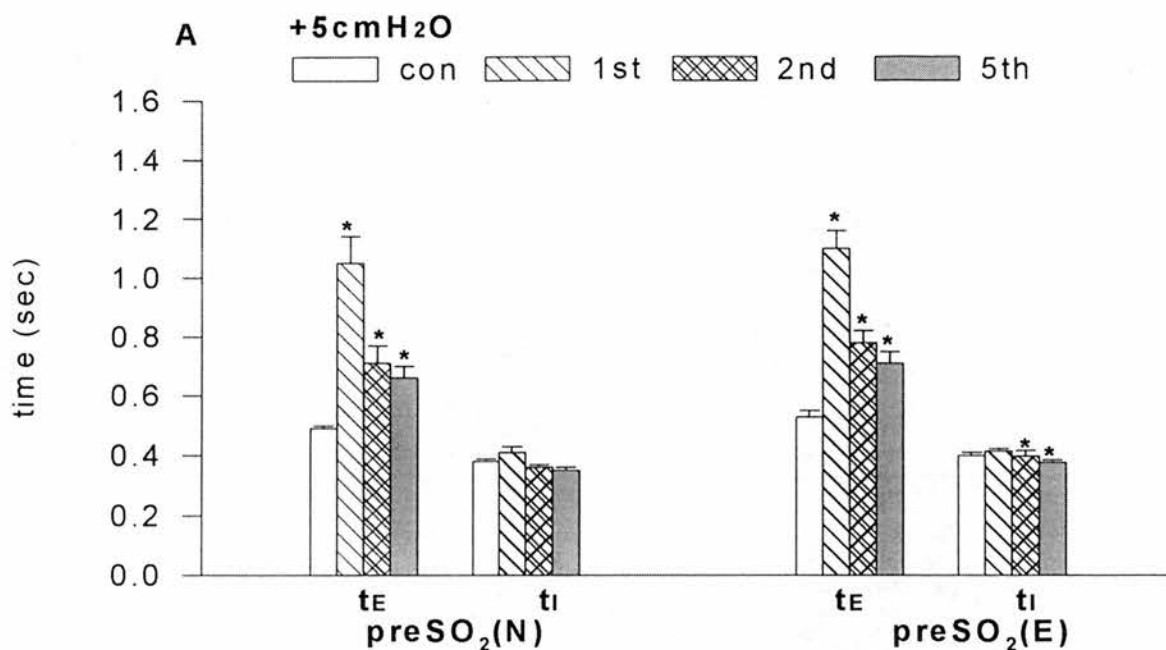
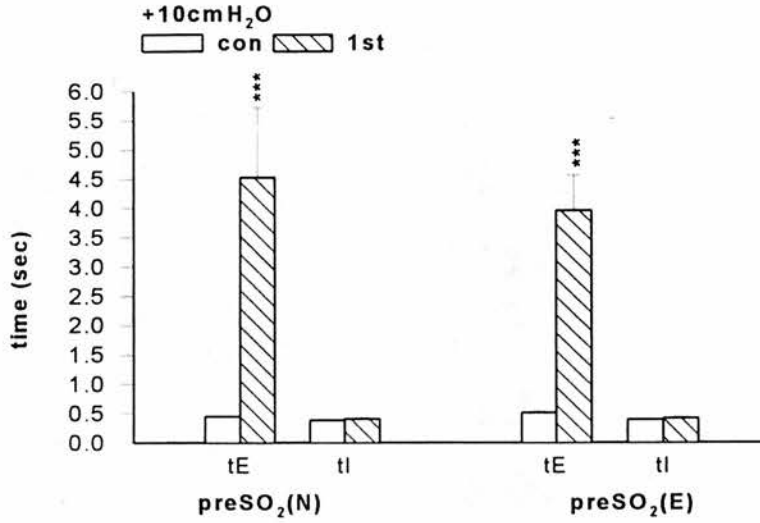


Fig. 3.11 Effect of inflation steps with +5cmH₂O on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were intact (preSO₂). Inspiratory time (ti), expiratory time (te), phrenic gradient (G), phrenic height (H). Data are mean±SEM. 1st, 2nd & 5th breaths under pressure vs. control breaths (con). * p≤0.05, ** p≤0.01 & *** p≤0.001.

A



B

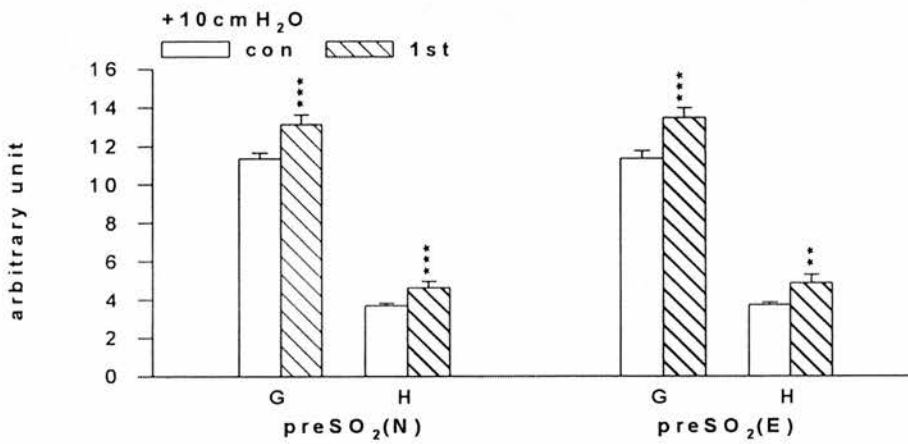


Fig. Effect of inflation steps with +10 cmH₂O on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were intact (preSO₂). Inspiratory time (tI), expiratory time (tE), phrenic gradient (G), phrenic height (H). Data are mean±SEM. 1st breath under pressure vs. control breaths (con). * p≤0.05, ** p≤0.01 & *** p≤0.001.

Comparison of the responses in normal and emphysematous rabbits

To facilitate comparison of variables between normal and emphysematous rabbits the 1st, 2nd and 5th breaths were calculated as % change from control breaths as follows:

$$tE_{1st, 2nd \text{ or } 5th} - tE_{control} / tE_{control} \cdot 100$$

The % change of emphysematous rabbits was compared to corresponding % change of normal using the Rank Sum Test.

Calculation of % change was performed in the same manner for comparison between normal and emphysematous rabbits for the rest of the results (inflation: postSO₂; deflation: preSO₂ & postSO₂).

There was a greater % change in tE of the 1st breath during HB inflation reflex in normal than emphysematous (Table 3.19A&B, Fig. 3.13A&B) rabbits for both +5 cm H₂O (N: 110.56±12.8; E: 97.12±7.1) and +10 cm H₂O (N: 840.18±236; E: 623.86±92.2), however these differences were not statistically significant. Inflation with +5 & +10 cm H₂O affected other variable (t_i , phrenic G & H) similarly in both normal and emphysematous rabbits (Table 3.19A&B, Fig. 3.13A&B).

Table 3.19 Comparison of pattern of breathing and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to an inflation step of + 5 (A) & +10 (B) cmH₂O, when SARs were intact (preSO₂). 1st, 2nd & 5th breaths were calculated as percentage change from control breath. Inspiratory time (ti), expiratory time (te), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

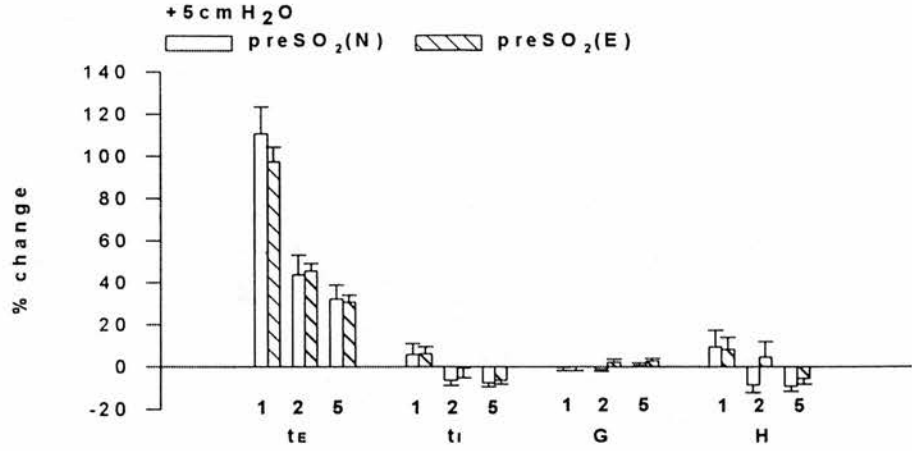
A

+5cmH ₂ O	PreSO ₂	1 st % change	2 nd % change	5 th % change
N(n=25)	te (N)	110.56±12.8	43.7±9.4	32±6.6
E(n=35)	te (E)	97.12±7.1	45.4±3.5	30.66±3.2
	ti (N)	5.8±5.1	-6.4±2.4	-7.8±1.6
	ti (E)	5.9±3.5	-0.54±4.6	-6.3±1.96
	G (N)	-0.29±1.47	-1.2±1.09	0.48±1.25
	G (E)	-0.16±1.6	1.99±1.5	2.6±1.4
	H (N)	9.46±7.64	-8.65±3.4	-9.04±2.4
	H (E)	8.2±5.7	4.5±7.3	-5.6±2.6

B

+10cmH ₂ O	PreSO ₂	1 st % change
N(n=25)	te (N)	840.18±236
E(n=35)	te (E)	623.86±92.2
	ti (N)	8.8±4.7
	ti (E)	9.53±5.01
	G (N)	16.07±4.2
	G (E)	19.97±4.47
	H (N)	26.07±8.59
	H (E)	31.53±9.9

A



B

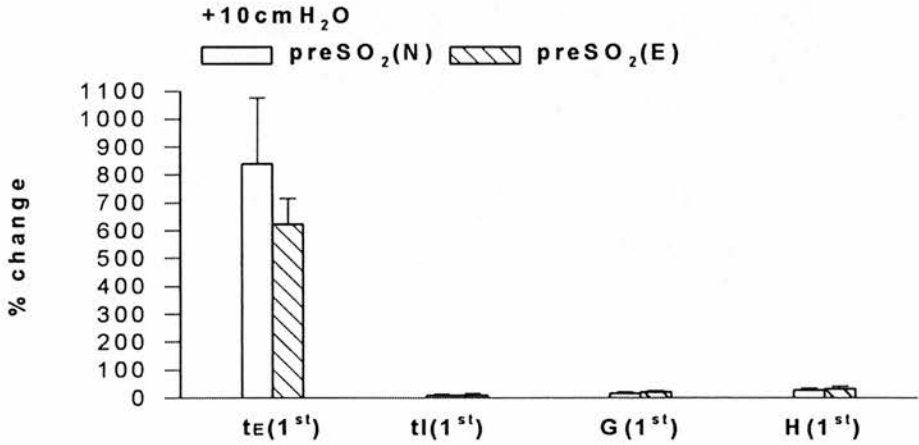


Fig. 3.13 Comparison of pattern of breathing and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to an inflation step of + 5 (A) & +10 (B) cmH₂O, when SARs were intact (preSO₂). 1st, 2nd & 5th breaths were calculated as percentage change from control breath. Inspiratory time (ti), expiratory time (tE), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

3.3.3.2 EFFECT OF INFLATION STEPS WITH 5 & 10 cmH₂O AFTER THE ADMINISTRATION OF SO₂ (POSTSO₂)

Effect of inflation steps in normal and emphysematous rabbits

In contrast to the marked lengthening of t_E with inflation before the administration of SO₂, after blockade of the SARs, inflations with 5 & 10 cm H₂O (table 3.20A&B, Fig. 3.14A&B, Fig. 3.15A&B), produced a reduction of t_E in both normal (+5: con: 0.63 ± 0.02 ; 1st: 0.45 ± 0.03 ; 2nd: 0.44 ± 0.03 ; 5th: 0.48 ± 0.02 . +10: con: 0.58 ± 0.02 ; 1st: 0.46 ± 0.04) and emphysematous rabbits (+5: con: 0.52 ± 0.02 ; 1st: 0.43 ± 0.02 ; 2nd: 0.42 ± 0.02 ; 5th: 0.41 ± 0.02 . +10: con: 0.48 ± 0.02 ; 1st: 0.41 ± 0.02) (Table 3.20, Fig. 3.14, 3.15).

With +5 cm H₂O inflation there were small but significant decreases in t_i in emphysematous rabbits in the 1st, 2nd & 5th breaths (Table 3.20A, Fig. 3.14A). However in normal rabbits although t_i decreased significantly for 2nd & 5th breaths, there was small significant increase in the 1st breath (con: 0.55 ± 0.01 ; 1st: 0.58 ± 0.02).

In contrast to the absence of effect on phrenic G prior to SO₂, postSO₂ inflation with +5 cm H₂O increased phrenic G of both normal (con: 8.45 ± 0.16 ; 1st: 8.71 ± 0.22 ; 2nd: 8.76 ± 0.23 ; 5th: 8.75 ± 0.2) and emphysematous rabbits (con: 11.06 ± 0.69 ; 1st: 11.62 ± 0.76 ; 2nd: 11.68 ± 0.76 ; 5th: 11.61 ± 0.74) (Table 3.20A, Fig. 3.14B). In normal rabbits, +5 cm H₂O decreased phrenic H of the 2nd & 5th breaths, however changes in phrenic H of emphysematous rabbits were not statistically significant (Table 3.20a, Fig. 3.14B).

As occurred prior to SO₂ administration, postSO₂ +10 cm H₂O increased phrenic G of both normal (con: 8.62 ± 0.17 ; 1st: 9.03 ± 0.24) and emphysematous rabbits (con: 11.38 ± 0.17 ; 1st: 11.79 ± 0.76), but it did not significantly change phrenic H of either normal or emphysematous rabbits (Table 3.20B, Fig. 3.15B).

Table 3.20 Effect of inflation steps with +5 (A) & +10 (B) cmH₂O on the pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were blocked with SO₂ (postSO₂). Inspiratory time (ti, sec), expiratory time (te, sec), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units). Data are mean±SEM. 1st, 2nd & 5th breaths under pressure vs. control breaths (con). * p≤0.05, ** p≤0.01 & *** p≤0.001.

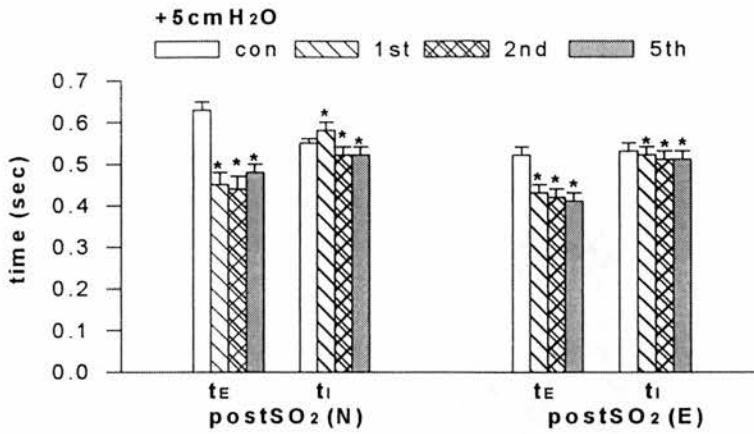
A

+5cmH ₂ O	PostSO ₂	con	1st	2nd	5th
N(n=25)	te	0.63±0.02	0.45±0.03 *	0.44±0.03 *	0.48±0.02 *
	ti	0.55±0.01	0.58±0.02 *	0.52±0.02 *	0.52±0.02 *
	G	8.45±0.16	8.71±0.22 *	8.76±0.23 *	8.75±0.2 *
	H	3.97±0.09	4.3±0.18	3.74±0.14 *	3.86±0.11*
E(n=35)	te	0.52±0.02	0.43±0.02 *	0.42±0.02 *	0.41±0.02 *
	ti	0.53±0.02	0.52±0.02 *	0.51±0.02 *	0.51±0.02 *
	G	11.06±0.69	11.62±0.76 *	11.68±0.76 *	11.61±0.74 *
	H	4.93±0.19	5.07±0.21	4.87±0.2	4.95±0.22

B

+10cmH ₂ O	PostSO ₂	con	1st
N(n=25)	te	0.58±0.02	0.46±0.04 ***
	ti	0.52±0.01	0.5±0.03
	G	8.62±0.17	9.03±0.24 ***
	H	3.84±0.06	3.68±0.16
E(n=35)	te	0.48±0.02	0.41±0.02 **
	ti	0.51±0.02	0.49±0.02
	G	11.38±0.17	11.79±0.76 **
	H	4.79±0.17	4.71±0.21

A



B

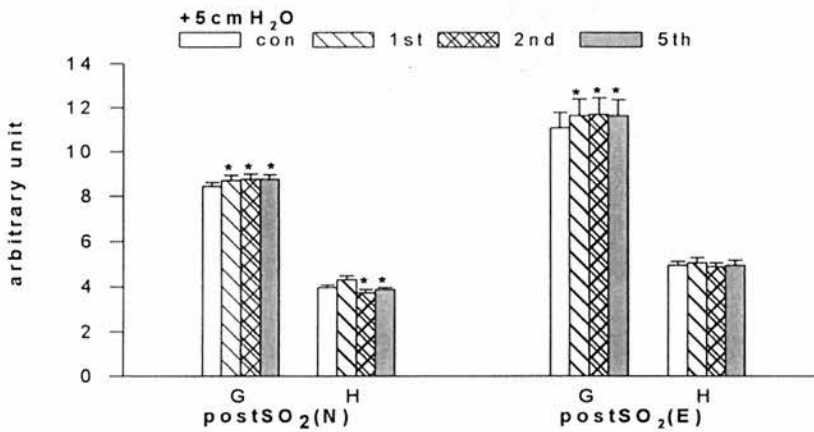
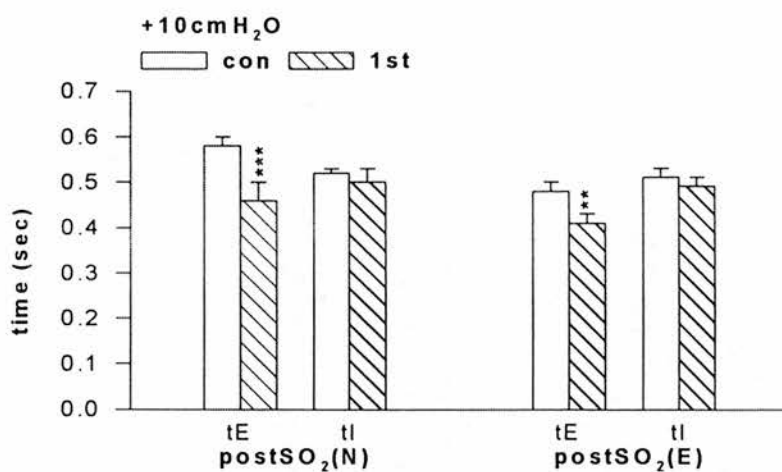


Fig. 3.14 Effect of inflation steps with +5 cmH₂O on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were blocked with SO₂ (postSO₂). Inspiratory time (t_I , sec), expiratory time (t_E , sec), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units). Data are mean \pm SEM. 1st, 2nd & 5th breaths under pressure vs. control breaths (con). * $p \leq 0.05$, ** $p \leq 0.01$ & *** $p \leq 0.001$.

A



B

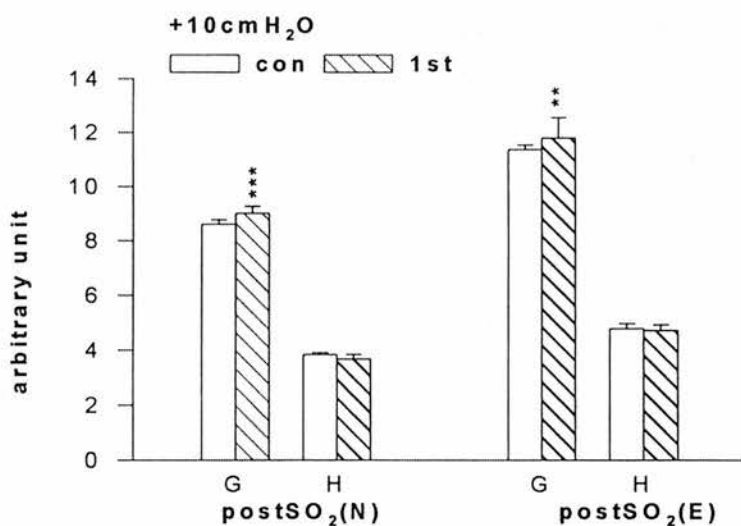


Fig. 3.15 Effect of inflation steps with +10 cmH₂O on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were blocked with SO₂ (postSO₂). Inspiratory time (tI, sec), expiratory time (tE, sec), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units). Data are mean±SEM. 1st breath under pressure vs. control breaths (con). * p≤0.05, ** p≤0.01 & *** p≤0.001

Comparison of the responses in normal and emphysematous rabbits

When considering the percentage change in particular variable (Table 3.21A, Fig. 3.16A) inflation with +5 cmH₂O reduced t_E more in normal (N) than emphysematous rabbits (E) in the 1st (N: -29.87 ± 2.97 ; E: -18.76 ± 2) and the 2nd breath (N: -31.8 ± 2.8 ; E: -19.27 ± 1.96). There was also a difference in the direction of change of t_I of the 1st breath during +5 cm H₂O inflation changed. There was a slight significant increase ($5.3 \pm 3.1\%$) in normal rabbits whereas in emphysematous rabbits there was a small but significant decrease ($-1.77 \pm 1.36\%$). However in the 2nd & 5th breaths there were no significant difference in t_I between normal and emphysematous rabbits. The changes in phrenic slope (G) & height (H) were not significantly different between the two groups. Inflation with +10 cm H₂O changed all variables (t_E , t_I , G, H) similarly in both normal and emphysematous rabbits (Table 3.21B, Fig. 3.16B).

Table 3.21 Comparison of pattern of breathing and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to + 5 (A) & +10 (B) cmH₂O, when SARs were blocked with SO₂ (postSO₂). 1st, 2nd & 5th breaths were calculated as percentage change from control breath. Inspiratory time (ti), expiratory time (te), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

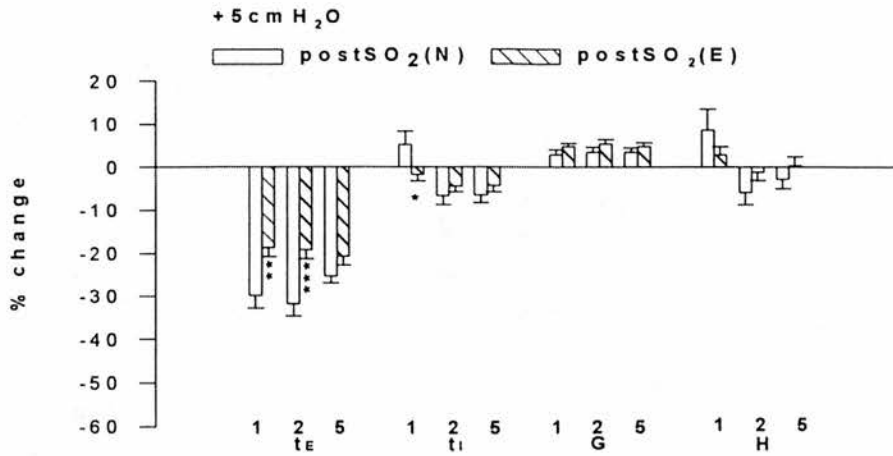
A

+5cmH ₂ O	PostSO ₂	1 st % change	2 nd % change	5 th % change
N(n=25)	te (N)	-29.87±2.97	-31.8±2.8	-25.2±1.63
E(n=35)	te (E)	-18.76±2 **	-19.27±1.96 **	-20.65±2.09
	ti (N)	5.3±3.1	-6.64±2	-6.46±1.81
	ti (E)	-1.77±1.36 *	-4.34±1.37	-4.25±1.5
	G (N)	2.9±1.1	3.5±1.2	3.5±1
	G (E)	4.8±0.8	5.4±1	4.8±0.9
	H (N)	8.7±4.9	-5.8±2.9	-2.8±2.2
	H (E)	2.9±1.9	-1.1±1.9	0.4±2.1

B

+10cmH ₂ O	PostSO ₂	1 st % change
N(n=25)	te (N)	-22.34±5.8
E(n=35)	te (E)	-14.04±4.89
	ti (N)	-6.24±3.3
	ti (E)	-3.06±2.46
	G (N)	4.54±1.01
	G (E)	3.41±1.16
	H (N)	-4.16±3.9
	H (E)	-1.81±2.58

A



B

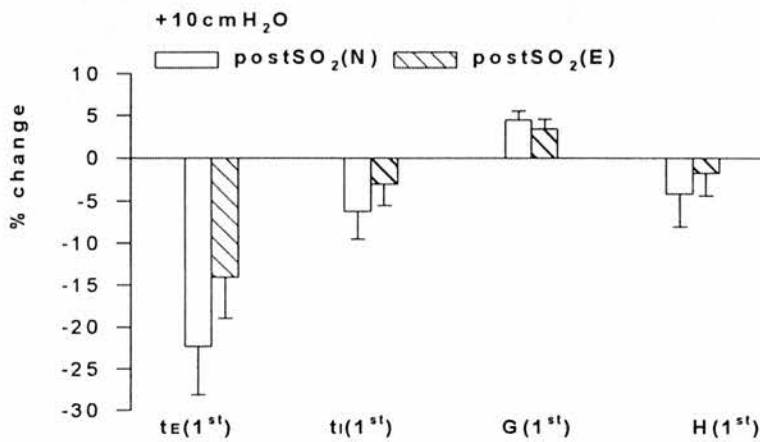


Fig. 3.16 Comparison of pattern of breathing and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to + 5 (A) & +10 (B) cmH₂O, when SARs were blocked with SO₂ (postSO₂). 1st, 2nd & 5th breaths were calculated as percentage change from control breath. Inspiratory time (t_I), expiratory time (t_E), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

3.3.4 EFFECT OF DEFLATION STEPS WITH 5 & 10 cmH₂O

3.3.4.1 EFFECT OF DEFLATION STEPS WITH 5 & 10 cmH₂O BEFORE THE ADMINISTRATION OF SO₂ (PRESO₂)

Effect of deflation steps in normal and emphysematous rabbits

In normal rabbits before SO₂ administration, measurement of the t_i of the 1st breath under 10 cm H₂O deflation revealed an increase in t_i greater than 1.8 times that of control breath in 18 out of 25 rabbits examined (Table 3.22B). These breaths were considered to be augmented breaths according to the criteria put forward by Davies & Roumy (1986). In emphysematous rabbits under the same condition 24 breaths out of 35 were augmented (Table 3.22B).

Deflation with 5 cm H₂O (Table 3.22A, Fig. 3.17A&B) shortened t_E of 1st 2nd & 5th breaths in normal (con: 0.47 ± 0.01 , 1st: 0.34 ± 0.02 , 2nd: 0.29 ± 0.01 , 5th: 0.32 ± 0.01) and emphysematous rabbits (con: 0.51 ± 0.02 , 1st: 0.35 ± 0.01 , 2nd: 0.34 ± 0.02 , 5th: 0.38 ± 0.02). Deflation with 5 cm H₂O increased phrenic slope (G) of the 1st 2nd & 5th breaths in normal (con: 11.38 ± 0.3 , 1st: 12.42 ± 0.37 , 2nd: 12.29 ± 0.34 , 5th: 12.49 ± 0.39) and emphysematous rabbits (con: 11.03 ± 0.37 , 1st: 11.76 ± 0.4 , 2nd: 12 ± 0.43 , 5th: 12.37 ± 0.45). Deflation with 5 cm H₂O increased phrenic height (H) of 1st 2nd & 5th breaths in normal (con: 3.71 ± 0.12 , 1st: 4.85 ± 0.21 , 2nd: 4.15 ± 0.1 , 5th: 4.13 ± 0.11) and emphysematous rabbits (con: 3.71 ± 0.12 , 1st: 4.61 ± 0.19 , 2nd: 4.32 ± 0.12 , 5th: 4.49 ± 0.15) (Table 3.22A, Fig. 3.17A&B).

Similarly 10 cm H₂O deflation shortened t_E , increased phrenic G & H in both normal and emphysematous rabbits (Table 3.22B, Fig. 3.18A&B). Deflation with 5 & 10 cm H₂O increased t_i of the 1st breath in both normal (-5 cm H₂O: con: 0.37 ± 0.05 vs. 1st: 0.43 ± 0.01 ; -10 cm H₂O: con: 0.37 ± 0.01 vs. 1st: 0.6 ± 0.02) and emphysematous rabbits (-5 cm H₂O: con: 0.39 ± 0.01 vs. 1st: 0.45 ± 0.01 ; -10 cm H₂O: con: 0.39 ± 0.01 vs. 1st: 0.61 ± 0.02), however the smaller increase in t_i of the 2nd & 5th breaths was only significant in emphysematous rabbits (Table 3.22A&B, Fig. 3.17A, 3.18A). The augmented breaths have been described above.

Table 3.22 Effect of deflation steps with -5 (A) & -10 (B) cmH₂O on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were intact (preSO₂). Inspiratory time (ti, sec), expiratory time (te, sec), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units). Data are mean±SEM. 1st, 2nd & 5th breaths under pressure vs. control breaths (con). * p≤0.05, ** p≤0.01 & *** p≤0.001.

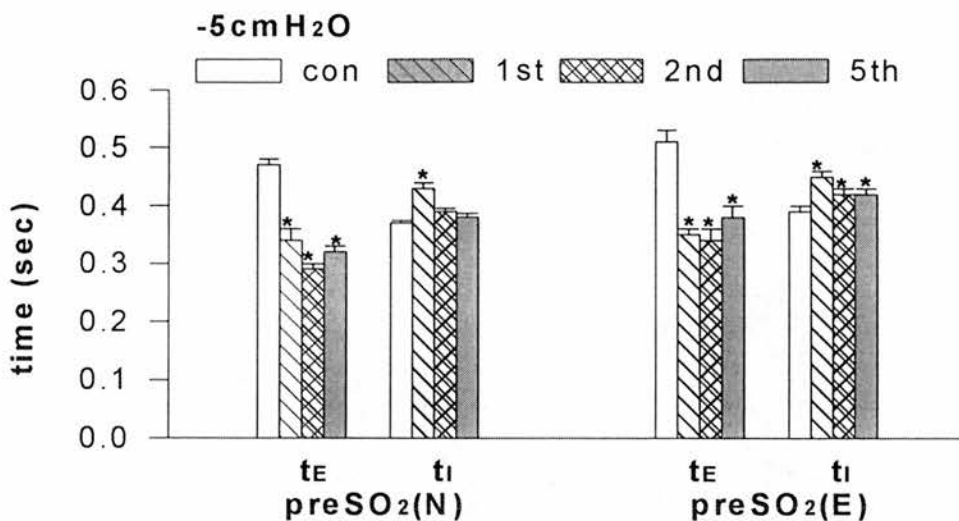
A

-5cmH ₂ O	PreSO ₂	con	1st	2nd	5th
N(n=25)	te	0.47±0.01	0.34±0.02 *	0.29±0.01 *	0.32±0.01 *
	ti	0.37±0.05	0.43±0.01 *	0.39±0.05	0.38±0.06
	G	11.38±0.3	12.42±0.37 *	12.29±0.34 *	12.49±0.39 *
	H	3.71±0.12	4.85±0.21 *	4.15±0.1 *	4.13±0.11 *
E(n=35)	te	0.51±0.02	0.35±0.01 *	0.34±0.02 *	0.38±0.02 *
	ti	0.39±0.01	0.45±0.01 *	0.42±0.01 *	0.42±0.01 *
	G	11.03±0.37	11.76±0.4 *	12±0.43 *	12.37±0.45 *
	H	3.71±0.12	4.61±0.19 *	4.32±0.12 *	4.49±0.15 *

B

-10cmH ₂ O	PreSO ₂	con	1st	2nd	5th
N(n=25)	te	0.44±0.01	0.32±0.02 *	0.22±0.01 *	0.25±0.01 *
	ti	0.37±0.01	0.6±0.02 *	0.37±0.01	0.35±0.05
	G	11.37±0.31	12.83±0.33 *	13.14±0.34 *	13.14±0.35 *
	H	3.65±0.12	7.1±0.33 *	4.2±0.15 *	4.06±0.11 *
E(n=35)	te	0.49±0.02	0.35±0.02 *	0.24±0.01 *	0.27±0.01 *
	ti	0.39±0.01	0.61±0.02 *	0.44±0.01 *	0.42±0.01 *
	G	11.4±0.38	12.7±0.43 *	13.04±0.43 *	13.13±0.42 *
	H	3.79±0.14	6.92±0.31 *	4.97±0.23 *	4.84±0.19 *

A



B

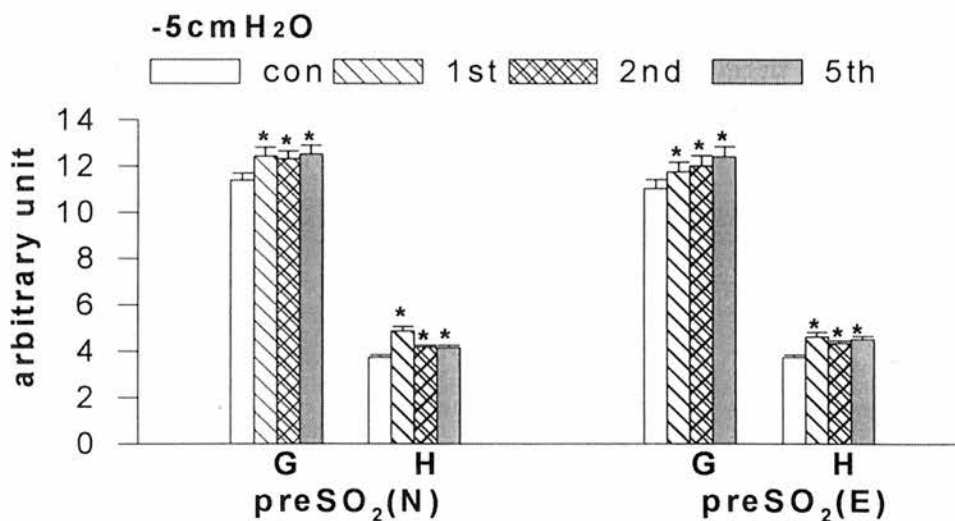
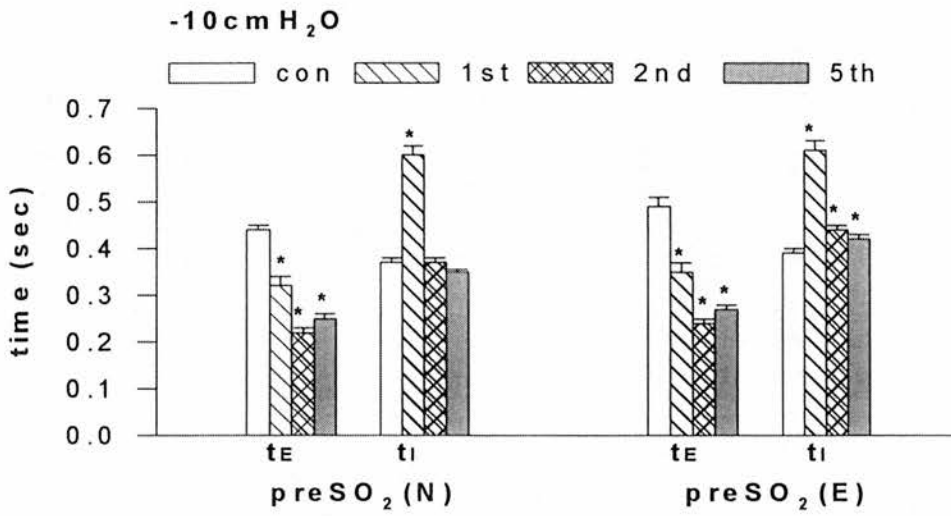


Fig. 3.17 Effect of deflation steps with $-5 \text{ cmH}_2\text{O}$ on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were intact (preSO₂). Inspiratory time (tI, sec), expiratory time (tE, sec), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units). Data are mean \pm SEM. 1st, 2nd & 5th breaths under pressure vs. control breaths (con). * $p \leq 0.05$, ** $p \leq 0.01$ & *** $p \leq 0.001$.

A



B

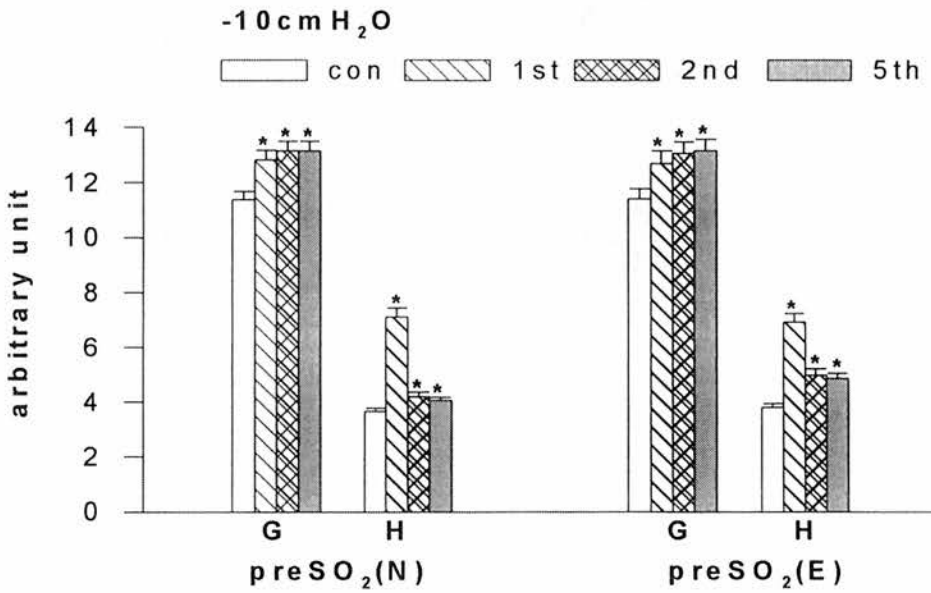


Fig. 3.18 Effect of deflation steps with $-10 \text{ cmH}_2\text{O}$ on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were intact (preSO₂). Inspiratory time (tI, sec), expiratory time (tE, sec), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units). Data are mean \pm SEM. 1st, 2nd & 5th breaths under pressure vs. control breaths (con). * $p \leq 0.05$, ** $p \leq 0.01$ & *** $p \leq 0.001$.

Comparison of the responses in normal and emphysematous rabbits

Deflation with steps of 5 & 10 cm H₂O changed most variables in a similar manner for both normal (N) and emphysematous (E) rabbits (Table 3.23A&B, Fig. 3.19A&B). However the t_i (N: $2.68 \pm 1.69\%$ vs. E: $7.83 \pm 1.35\%$) of the 5th breath under a deflation pressure of -5 cm H₂O was slightly increased in emphysematous rabbits and did not change in normal, a difference that was statistically significant. The increase in phrenic H of 5th breath under 5 cm H₂O deflation pressure was greater in emphysematous (21.55 ± 2.16) than in normal rabbits ($11.99 \pm 2.37\%$). Similarly the t_i of the 2nd & 5th under -10 cm H₂O deflation pressure were increased in emphysematous rabbits (2nd: $13.24 \pm 2.49\%$, 5th: $8.24 \pm 1.9\%$) but did not change in normal (2nd: $0.24 \pm 3.4\%$; 5th: $-4.4 \pm 2.6\%$), a difference that was statistically significant. The increase in phrenic H of the 2nd & 5th breath under 10 cm H₂O deflation pressure was greater in emphysematous (2nd: 32.25 ± 4.27 , 5th: 29.53 ± 4.06) than in normal rabbits (2nd: 16.53 ± 4.45 , 5th: 12.46 ± 3.42) (Table 3.23, Fig. 3.19).

Table 3.23 Comparison of pattern of breathing and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to - 5 (A) & -10 (B) cmH₂O, when SARs were intact (preSO₂). 1st, 2nd & 5th breaths were calculated as percentage change from control breath. Inspiratory time (ti), expiratory time (te), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

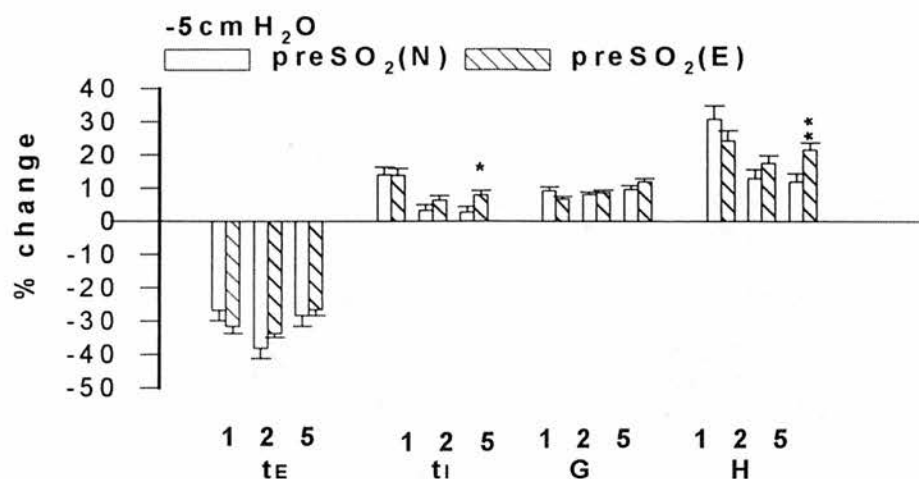
A

-5cmH ₂ O	PreSO ₂	1 st % change	2 nd % change	5 th % change
N(n=25)	te (N)	-26.8±2.98	-38.1±3.2	-28.2±3.4
E(n=35)	te (E)	-31.64±2.12	-33.72±1.17	-26.61±1.6
	ti (N)	14.1±2.3	3.3±1.7	2.68±1.69
	ti (E)	13.77±2.11	6.38±1.37	7.83±1.35 *
	G (N)	9.2±1.2	8.07±0.89	9.64±1.15
	G (E)	6.7±0.74	8.7±0.79	11.98±0.94
	H (N)	30.75±4.03	12.9±2.73	11.99±2.37
	H (E)	24.26±3.07	17.56±2.22	21.55±2.16 *

B

-10cmH ₂ O	PreSO ₂	1 st % change	2 nd % change	5 th % change
N(n=25)	te (N)	-27.3±2.9	-49.97±2.1	-40.3±3.01
E(n=35)	te (E)	-28.22±2.61	-48.83±1.62	-43.68±1.49
	ti (N)	62.4±4.8	0.24±3.4	-4.4±2.6
	ti (E)	57.61±4.1	13.24±2.49 *	8.24±1.9 *
	G (N)	12.97±0.96	15.65±0.79	15.78±1.5
	G (E)	11.49±0.84	14.62±1.03	15.57±1.25
	H (N)	94.8±6.97	16.53±4.45	12.46±3.42
	H (E)	84.1±5.86	32.25±4.27 *	29.53±4.06 *

A



B

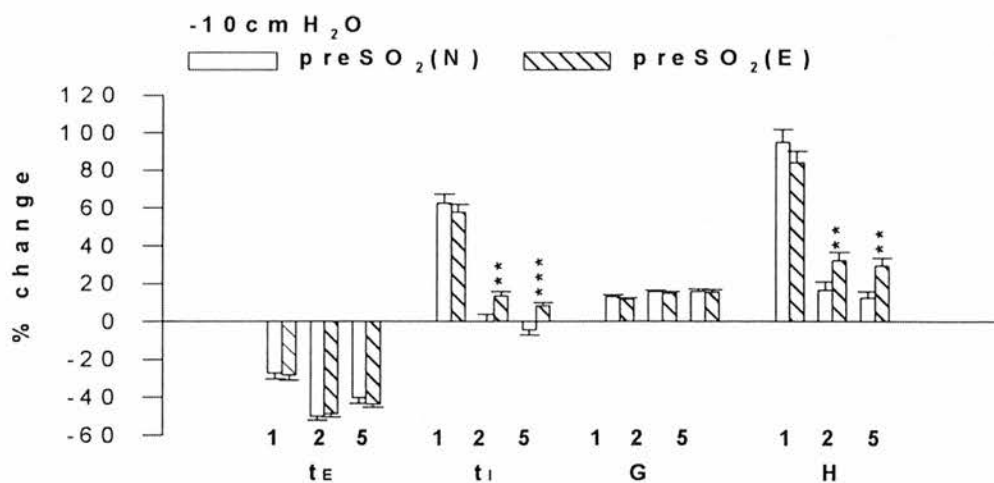


Fig. 3.19 Comparison of pattern of breathing and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to - 5 (A) & -10 (B) cmH₂O, when SARs were intact (preSO₂). 1st, 2nd & 5th breaths were calculated as percentage change from control breath. Inspiratory time (tI), expiratory time (tE), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p<0.05, ** p<0.01 & *** p<0.001.

3.3.4.2 EFFECT OF DEFLATION STEPS WITH 5 & 10 cmH₂O AFTER THE ADMINISTRATION OF SO₂ (POSTSO₂)

Effect of deflation steps in normal and emphysematous rabbits

After SO₂ administration, the number of augmented breaths under 10 cm H₂O deflation was decreased in both normal and emphysematous rabbits. In normal rabbits there were two augmented breaths out of 25, and in emphysematous rabbits there were ten augmented breaths out of 35.

Deflation with 5 cm H₂O increased phrenic H of the 1st, 2nd & 5th breaths in normal and emphysematous rabbits, however this increase was only associated with a significant increase in t_i in emphysematous rabbits (Table 3.24A, Fig. 3.20). The stronger stimulus 10 cm H₂O deflation pressure increased phrenic H of the 1st, 2nd & 5th breaths in normal and emphysematous rabbits, and this was associated with a significant increase in t_i in both normal and emphysematous rabbits (Table 3.24B, Fig. 3.21). Deflation with 5 & 10 cm H₂O increased phrenic G in both normal and emphysematous rabbits. However, the increase in phrenic G of the 2nd breath of normal rabbits for 5cm H₂O deflation was not statistically significant (Table 3.24A). In normal rabbits, 5 & 10 cm H₂O deflation decreased t_E of the 1st, however that of 2nd & 5th breath were only reduced with -10 cm H₂O. In emphysematous rabbits, 5 cm H₂O deflation significantly decreased t_E of 1st breath, and significantly increased that of 5th breath, however that of 2nd was not statistically changed. In emphysematous rabbits 10 cm H₂O deflation did not statistically change t_E of the 1st, 2nd & 5th breaths. (Table 3.24A&B, Fig. 3.20A&B, 3.21A&B)

Table 3.24 Effect of deflation steps with -5 (A) & -10 (B) cmH₂O on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were blocked with SO₂ (postSO₂). Inspiratory time (ti, sec), expiratory time (te, sec), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units). Data are mean±SEM. 1st, 2nd & 5th breaths under pressure vs. control breaths (con). * p≤0.05, ** p≤0.01 & *** p≤0.001.

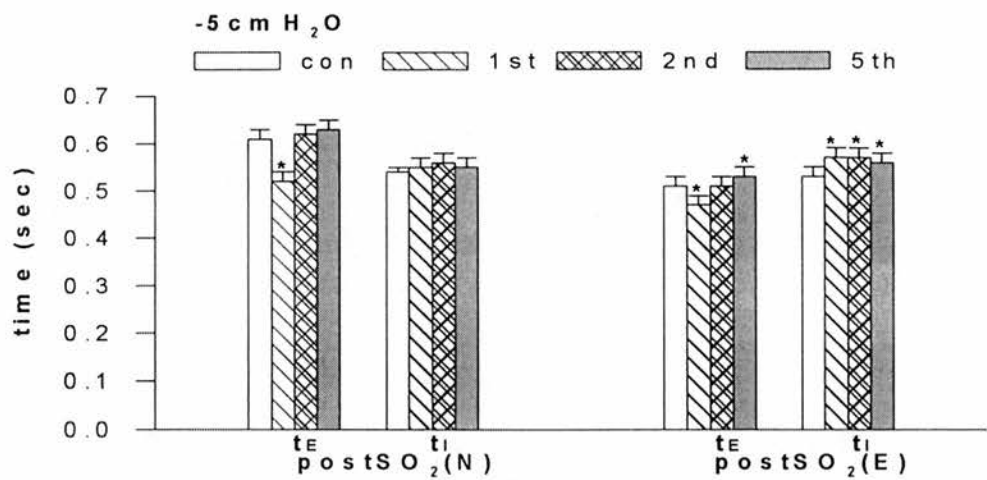
A

-5cmH ₂ O	PostSO ₂	con	1st	2nd	5th
N(n=25)	te	0.61±0.02	0.52±0.02 *	0.62±0.02	0.63±0.02
	ti	0.54±0.01	0.55±0.02	0.56±0.02	0.55±0.02
	G	8.57±0.19	8.82±0.17 *	8.59±0.18	8.91±0.11 *
	H	3.89±0.07	4.23±0.1 *	4.2±0.15 *	4.22±0.13 *
E(n=35)	te	0.51±0.02	0.47±0.02 *	0.51±0.02	0.53±0.02 *
	ti	0.53±0.02	0.57±0.02 *	0.57±0.02 *	0.56±0.02 *
	G	11.36±0.75	11.69±0.78 *	11.59±0.77 *	11.96±0.8 *
	H	5.04±0.2	5.67±0.25 *	5.5±0.21 *	5.59±0.23 *

B

-10cmH ₂ O	PostSO ₂	con	1st	2nd	5th
N(n=25)	te	0.56±0.01	0.44±0.01 *	0.51±0.02 *	0.53±0.02 *
	ti	0.52±0.01	0.56±0.02 *	0.57±0.02 *	0.55±0.02 *
	G	8.61±0.18	9.11±0.14 *	9.03±0.15 *	9.16±0.13 *
	H	3.8±0.06	4.31±0.19 *	4.39±0.16 *	4.34±0.16 *
E(n=35)	te	0.46±0.02	0.42±0.01	0.45±0.02	0.46±0.02
	ti	0.51±0.02	0.64±0.03 *	0.59±0.02 *	0.58±0.02 *
	G	11.42±0.75	12±0.77 *	12.09±0.81 *	12.18±0.78 *
	H	4.81±0.17	6.38±0.3 *	6.03±0.19 *	5.98±0.21 *

A



B

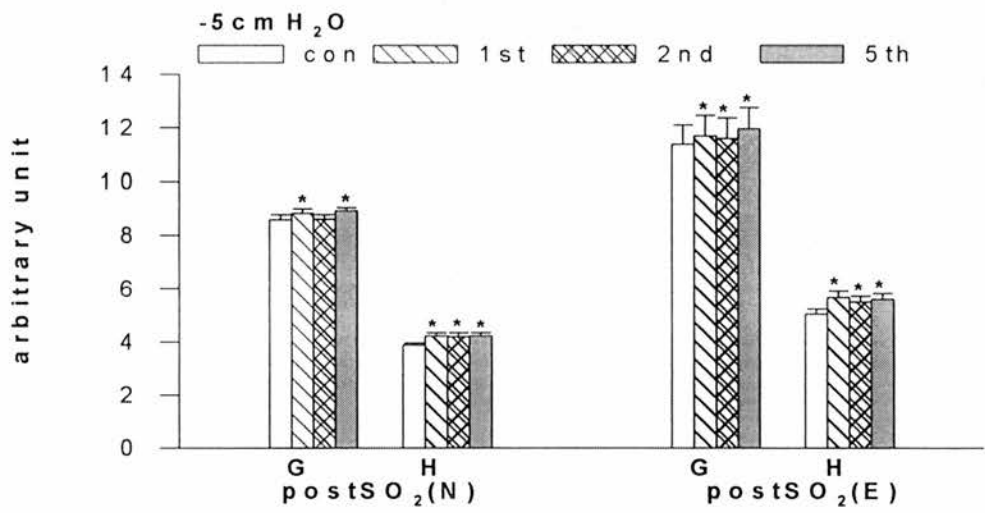
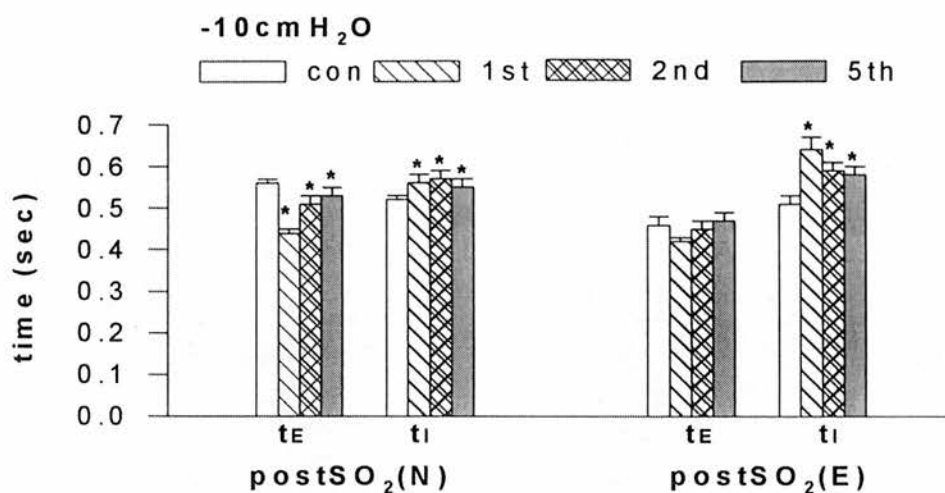


Fig. 3.20 Effect of deflation steps with $-5 \text{ cmH}_2\text{O}$ on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were blocked with SO_2 (post SO_2). Inspiratory time (t_I , sec), expiratory time (t_E , sec), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units). Data are mean \pm SEM. 1st, 2nd & 5th breaths under pressure vs. control breaths (con). * $p \leq 0.05$, ** $p \leq 0.01$ & *** $p \leq 0.001$.

A



B

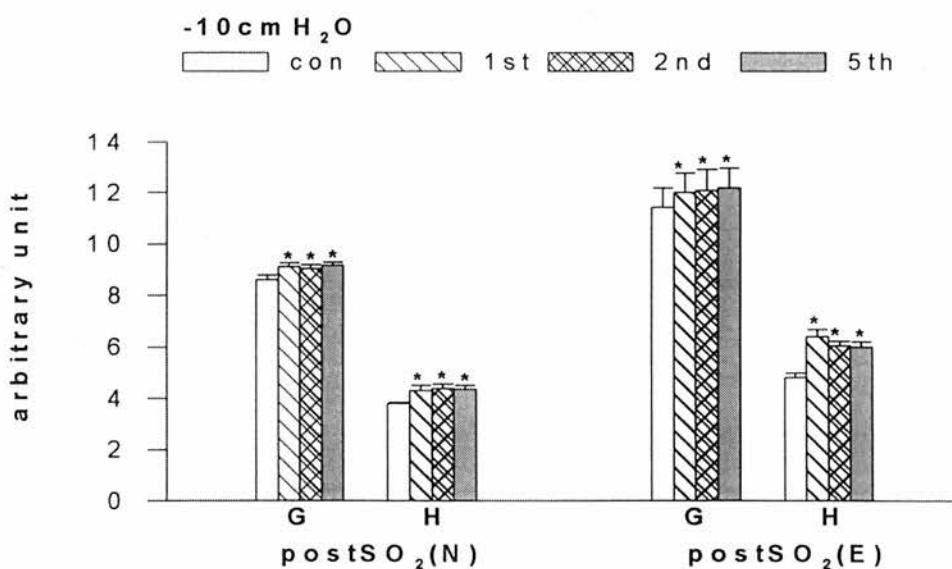


Fig. 3.21 Effect of deflation steps with $-10 \text{ cmH}_2\text{O}$ on pattern of breathing and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were blocked with SO_2 (post SO_2). Inspiratory time (tI, sec), expiratory time (tE, sec), phrenic gradient (G, computer arbitrary units), phrenic height (H, computer arbitrary units). Data are mean \pm SEM. 1st, 2nd & 5th breaths under pressure vs. control breaths (con). * $p \leq 0.05$, ** $p \leq 0.01$ & *** $p \leq 0.001$.

Comparison of the responses in normal and emphysematous rabbits

Deflation with 5 cm H₂O produced the same directional change for all variables: t_E , t_I , phrenic G & H of 1st, 2nd & 5th breaths (except for t_E of the 2nd breath) in normal and emphysematous rabbits. However, deflation with 10 cm H₂O increased t_I (1st: N: 6.91 ± 2.88 vs. E: 23.59 ± 3.37 ; 2nd: N: 9.24 ± 2.23 vs. E: 15.67 ± 1.31 ; 5th: N: 5.63 ± 2.03 vs. E: 14.13 ± 1.68) and phrenic H (1st: N: 13.52 ± 4.39 vs. E: 32.6 ± 4.27 ; 2nd: N: 15.76 ± 3.59 vs. E: 25.93 ± 2.02 ; 5th: N: 14.36 ± 3.71 vs. E: 24.74 ± 2.14) of the 1st, 2nd & 5th breaths more in emphysematous rabbits than in normal rabbits (Tables 3.25B, Fig. 3.22B). Deflation with 10 cm H₂O decreased t_E of 1st breath more in normal (N: -20.26 ± 2.23 vs. E: -7.86 ± 2.39) as compared to emphysematous animals. However, that of 2nd & 5th breaths were changed equally in both normal and emphysematous rabbits (Table 3.25, Fig. 3.22).

Table 3.25 Comparison of pattern of breathing and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to a step deflation of -5 (A) & -10 (B) cmH₂O, when SARs were blocked with SO₂ (postSO₂). 1st, 2nd & 5th breaths were calculated as percentage change from control breath. Inspiratory time (ti), expiratory time (te), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

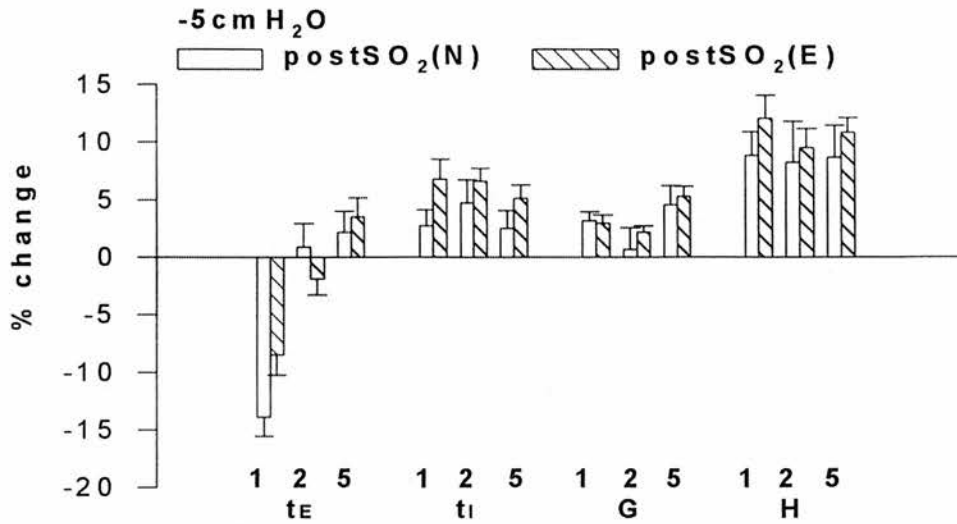
A

-5cmH ₂ O	PostSO ₂	1 st % change	2 nd % change	5 th % change
N(n=25)	te (N)	-13.91±1.63	0.9±1.99	2.16±1.79
E(n=35)	te (E)	-8.47±1.77	-1.88±1.41	3.47±1.69
	ti (N)	2.69±1.39	4.66±2.05	2.46±1.59
	ti (E)	6.72±1.73	6.59±1.09	5.05±1.2
	G (N)	3.16±0.75	0.63±1.88	4.51±1.66
	G (E)	2.92±0.71	2.14±0.57	5.25±0.88
	H (N)	8.81±2.02	8.2±3.52	8.65±2.72
	H (E)	12.03±1.98	9.45±1.66	10.8±1.27

B

-10cmH ₂ O	PostSO ₂	1 st % change	2 nd % change	5 th % change
N(n=25)	te (N)	-20.26±2.23	-7.95±2.14	-4.69±1.72
E(n=35)	te (E)	-7.86±2.39 **	-2.84±1.66	0.05±1.93
	ti (N)	6.91±2.88	9.24±2.23	5.63±2.03
	ti (E)	23.59±3.37 **	15.67±1.31 **	14.13±1.68 **
	G (N)	6.14±1.13	5.11±1.24	6.92±1.74
	G (E)	5.18±0.77	5.63±0.73	6.82±0.75
	H (N)	13.52±4.39	15.76±3.59	14.36±3.71
	H (E)	32.6±4.27 **	25.93±2.02 *	24.74±2.14 **

A



B

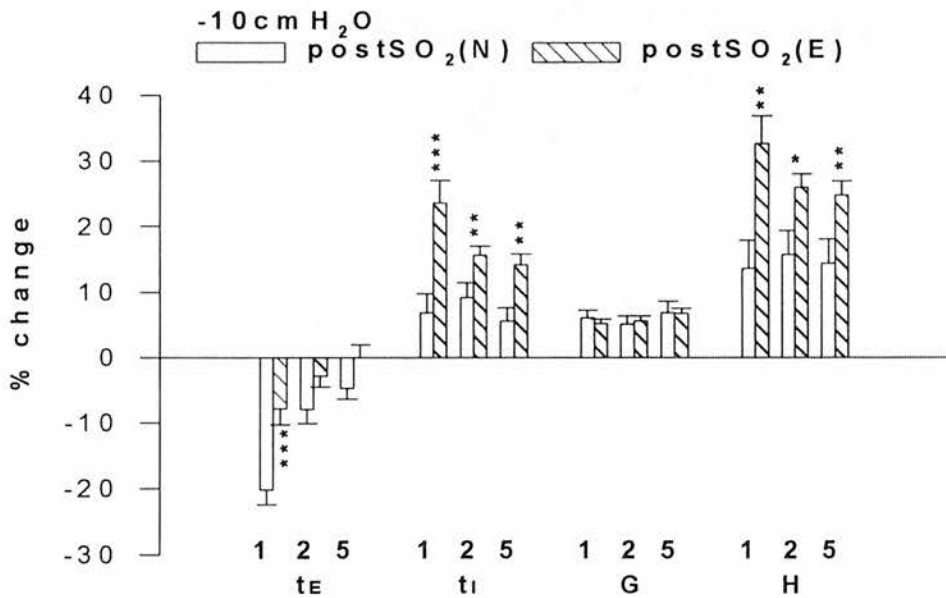


Fig. 3.22 Comparison of pattern of breathing and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to -5 (A) & -10 (B) cmH₂O, when SARs were blocked with SO₂ (postSO₂). 1st, 2nd & 5th breaths were calculated as percentage change from control breath. Inspiratory time (tI), expiratory time (tE), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

3.3.5 EFFECT OF 4 & 6 % CO₂ ON PATTERN OF BREATHING, VENTILATION AND PHRENIC ACTIVITY

3.3.5.1 EFFECT OF 4 & 6 % CO₂ ON PATTERN OF BREATHING, VENTILATION AND PHRENIC ACTIVITY BEFORE ADMINISTRATION OF SO₂

Effect of CO₂ in normal and emphysematous rabbits

The effects of 4 & 6% CO₂ were investigated by causing the rabbits to breathe 4 % CO₂ in oxygen for two minutes, and 6% CO₂ in oxygen for another two minutes. To examine the effect of CO₂ on the measured variables the mean of 5 breaths was measured a) immediately before CO₂, b) just before the termination of 4% CO₂ and c) just before the termination of 6% CO₂. The variables analysed were: expiratory time (te, sec), inspiratory time (ti, sec), tidal volume (V_T, ml), minute ventilation (\dot{V}_I , l/min), frequency of breathing (f, breaths/min), phrenic gradient (G, computer arbitrary units), and phrenic height (H, computer arbitrary units). Data are mean \pm SEM and statistical significance was set at $p \leq 0.05$.

To determine whether or not CO₂ affected the above variables, the value for the particular variable during 4 & 6% CO₂ inhalation was compared to the value of the variable for the control breaths immediately prior to CO₂ inhalation, using Repeated Measures ANOVA on Ranks.

In normal and emphysematous rabbits 4% CO₂ decreased t_i and this was further decreased by 6% CO₂ (Table 3.26, Fig. 3.23A). t_e was also decreased in both groups for the two CO₂ concentrations but this decrease did not reach significance in normal rabbits with 4% CO₂. Thus for all the rabbits, there were significant increases in breathing frequency with both levels of CO₂. In normal and emphysematous rabbits 4% CO₂ caused an increase in V_T and this was further increased with 6% CO₂. This increase in V_T was associated with an increase in phrenic height (H) but this only reached significance in the emphysematous rabbits. Phrenic slope (G) was significantly increased after CO₂ for normal and emphysematous animals, the increases with 6% CO₂ inhalation being greater than those with 4% CO₂ inhalation. As CO₂ increased

both breathing frequency and V_T , \dot{V}_I was significantly increased in all cases, with greater increases when the CO_2 concentration was 6% (Table 3.26, Fig. 3.23A&B).

Table 3.26 Effect of 4 & 6% CO₂ on pattern of breathing, ventilation and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were intact (preSO₂). Expiratory time (t_E, sec), inspiratory time (t_I, sec), tidal volume (V_T, ml), minute ventilation (\dot{V}_I , l/min), frequency of breathing (f, breaths/min), phrenic gradient (G, computer arbitrary units), and phrenic height (H, computer arbitrary units). 4 & 6% CO₂ vs. control breath (con). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

4 & 6% CO ₂	PreSO ₂	con	4% CO ₂	6% CO ₂
N(n=25)	t _E	0.49±0.01	0.43±0.01	0.38±0.01 *
	t _I	0.39±0.01	0.35±0.01 *	0.33±0.01 *
	f	69.43±1.26	77.6±1.82 *	86.53±2.23 *
	V _T	10.32±0.46	15.89±0.72 *	16.88±0.77 *
	\dot{V}_I	0.72±0.04	1.2±0.07 *	1.5±0.08 *
	G	10.41±0.23	12.52±0.15 *	13.47±0.15 *
	H	3.48±0.1	3.75±0.11	3.69±0.14
E(n=35)	t _E	0.52±0.02	0.44±0.01 *	0.38±0.01 *
	t _I	0.4±0.01	0.35±0.01 *	0.32±0.01 *
	f	66.32±1.13	77.07±1.66 *	88.8±2.28 *
	V _T	12.68±0.27	21.16±1.2 *	21.88±1.01 *
	\dot{V}_I	0.8±0.01	1.6±0.07 *	1.9±0.05 *
	G	11.35±0.32	13.81±0.4 *	15.54±0.41 *
	H	3.89±0.11	4.1±0.09 *	4±0.09 *

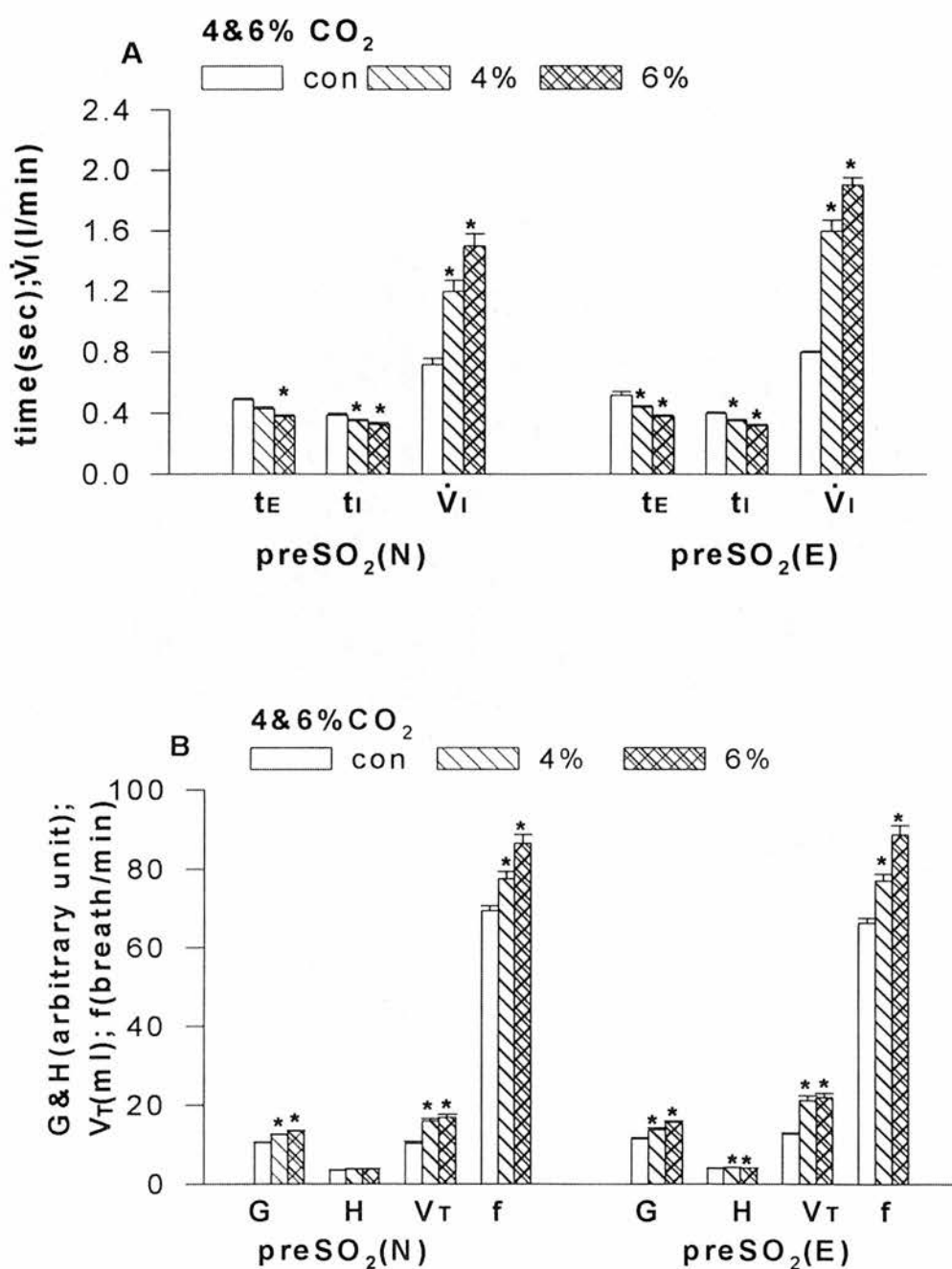


Fig. 3.23 Effect of 4 & 6% CO₂ on pattern of breathing, ventilation and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were intact (preSO₂). Inspiratory time (t_I), expiratory time (t_E), frequency of breathing (f), tidal volume (V_T), minute ventilation (\dot{V}_I), phrenic gradient (G), phrenic height (H). 4 & 6% CO₂ vs. control breaths (con). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

Comparison of the responses in normal and emphysematous rabbits

To facilitate comparison between normal and emphysematous rabbit, the response to 4 & 6% CO₂ was calculated as the % change from the control breath as follows:

$$tE_{4 \& 6\% CO_2} - tE_{control} / tE_{control} \cdot 100$$

The % change of emphysema (E) was compared to corresponding % change of normal (N) using the Rank Sum Test.

Calculation of % change was performed in the same manner for comparison between normal and emphysematous rabbits for the rest of the results (postSO₂ & postvagotomy).

Inhalation of 4% CO₂ changed all variables (tE , tI , f , V_T , \dot{V}_I , phrenic G & H) in both emphysematous and normal rabbits to a similar degree (Table 3.27, Fig. 3.24). Inhalation of 6% CO₂, however, reduced tI more in emphysematous rabbits (N: -15.9 ± 1.3 ; E: -20.2 ± 1.2) and thus f was increased significantly more in emphysematous rabbits. 6% CO₂ reduced tE more in emphysematous animals, however, this did not reach significance ($p=0.06$). 6% CO₂ inhalation increased V_T in both emphysematous and normal animals to a similar degree, however \dot{V}_I was increased significantly more in emphysematous rabbits breathing 6% CO₂, due to the greater increase in f . 6% CO₂ inhalation increased phrenic G significantly more in emphysematous rabbits than normal animals. There was no significant difference between the phrenic H of emphysematous and normal rabbits, although the increase in phrenic H had reached significance only in emphysematous rabbits at both levels of CO₂ (table 3.27, Fig. 3.24).

Table 3.27 Comparison of pattern of breathing, ventilation and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to 4 & 6% CO₂, when SARs were intact (preSO₂). 4 & 6% CO₂ were calculated as percentage change from control breath. Inspiratory time (ti), expiratory time (te), frequency of breathing (f), tidal volume (VT), minute ventilation (V̇I), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

4 & 6% CO ₂	PreSO ₂	4% CO ₂ % change	6% CO ₂ % change
N(n=25)	te (N)	-10.6±2.6	-21.7±2.6
E(n=35)	te (E)	-15±1.2	-27.9±1
	ti (N)	-8.8±1.3	-15.9±1.3
	ti (E)	-11.4±1	-20.2±1.2 *
	f (N)	12±2.1	24.9±2.7
	f (E)	16.1±1.1	33.4±1.6 *
	VT (N)	55.9±6	70.3±9.2
	VT (E)	63.5±5.8	70.4±4.7
	V̇I (N)	74.8±7.7	111.6±11.4
	V̇I (E)	89.1±6.3	126.2±5.5 *
	G (N)	21.3±2.3	30.9±3
	G (E)	21.8±1.2	37.6±1.8 *
	H (N)	8.3±2.3	6.2±3.4
	H (E)	6.4±1.5	4.1±2

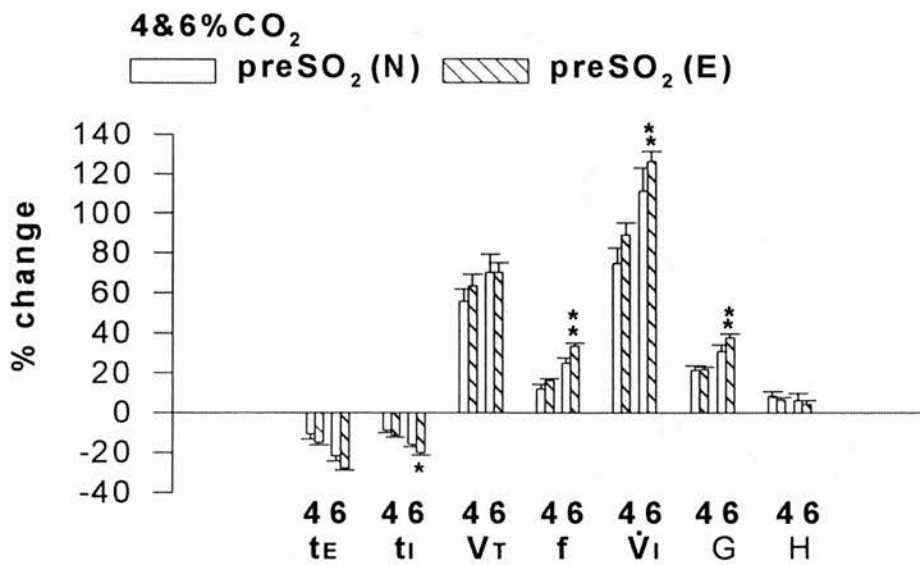


Fig. 3.24 Comparison of pattern of breathing, ventilation and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to 4 & 6% CO₂, when SARs were intact (preSO₂). 4 & 6% CO₂ were calculated as percentage change from control breath. Inspiratory time (ti), expiratory time (tE), frequency of breathing (f), tidal volume (VT), minute ventilation (V̇I), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

3.3.5.2 EFFECT OF 4 & 6 % CO₂ ON PATTERN OF BREATHING, VENTILATION AND PHRENIC ACTIVITY AFTER ADMINISTRATION OF SO₂

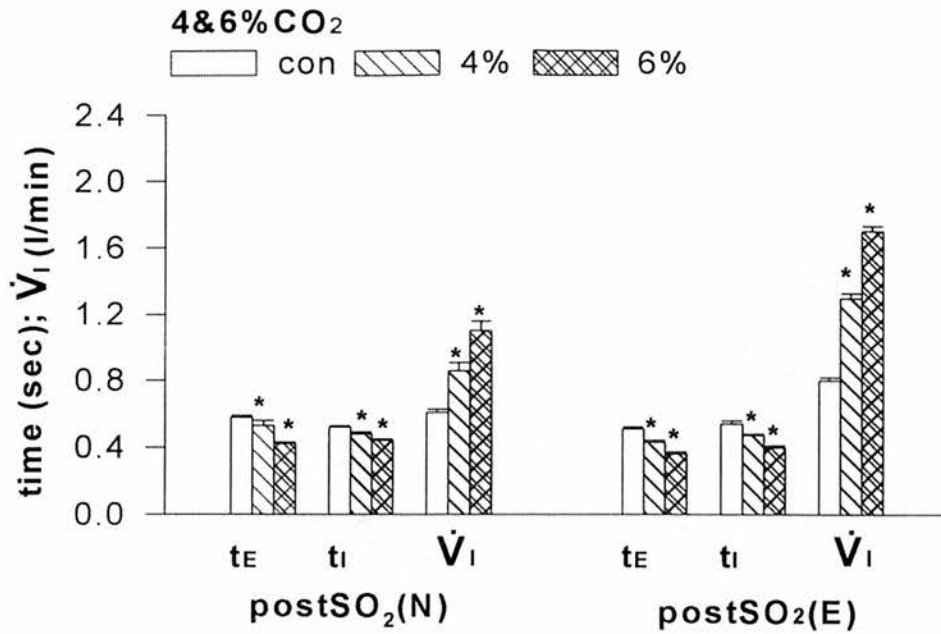
Effect of CO₂ in normal and emphysematous rabbits

In normal and emphysematous rabbits, 4 & 6% CO₂ inhalation decreased t_E & t_I significantly, thus f was significantly increased (Table 3.28, Fig. 3.25A&B). CO₂ inhalation increased V_T significantly, and thus \dot{V}_I was significantly increased. CO₂ inhalation significantly increased phrenic G, however phrenic H was not affected (Table 3.28, Fig. 3.25A&B).

Table 3.28 Effect of 4 & 6% CO₂ on pattern of breathing, ventilation and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were blocked with SO₂ (postSO₂). Expiratory time (t_E, sec), inspiratory time (t_I, sec), tidal volume (V_T, ml), minute ventilation (\dot{V}_I , l/min), frequency of breathing (f, breaths/min), phrenic gradient (G, computer arbitrary units), and phrenic height (H, computer arbitrary units). 4 & 6% CO₂ vs. control breaths (con). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

4 & 6% CO ₂	PostSO ₂	con	4% CO ₂	6% CO ₂
N(n=25)	t _E	0.58±0.01	0.53±0.03 *	0.42±0.01 *
	t _I	0.52±0.01	0.48±0.01 *	0.44±0.01 *
	f	54.61±0.59	60.28±1.59 *	70.11±1.8 *
	V _T	11.23±0.42	13.98±0.47 *	15.65±0.55 *
	\dot{V}_I	0.61±0.02	0.86±0.05 *	1.1±0.06 *
	G	9.95±0.22	11.51±0.37 *	12.33±0.36 *
	H	4.4±0.13	4.75±0.18	4.8±0.25
E(n=35)	t _E	0.51±0.01	0.43±0.01 *	0.36±0.01 *
	t _I	0.54±0.02	0.47±0.01 *	0.4±0.01 *
	f	59.23±1.8	67.93±1.47 *	80.75±2.01 *
	V _T	13.83±0.34	20.3±0.79 *	21.28±0.66 *
	\dot{V}_I	0.8±0.02	1.3±0.03 *	1.7±0.03 *
	G	11.31±0.73	13.23±0.7 *	14.99±0.87 *
	H	5.05±0.2	5.15±0.2	4.93±0.29

A



B

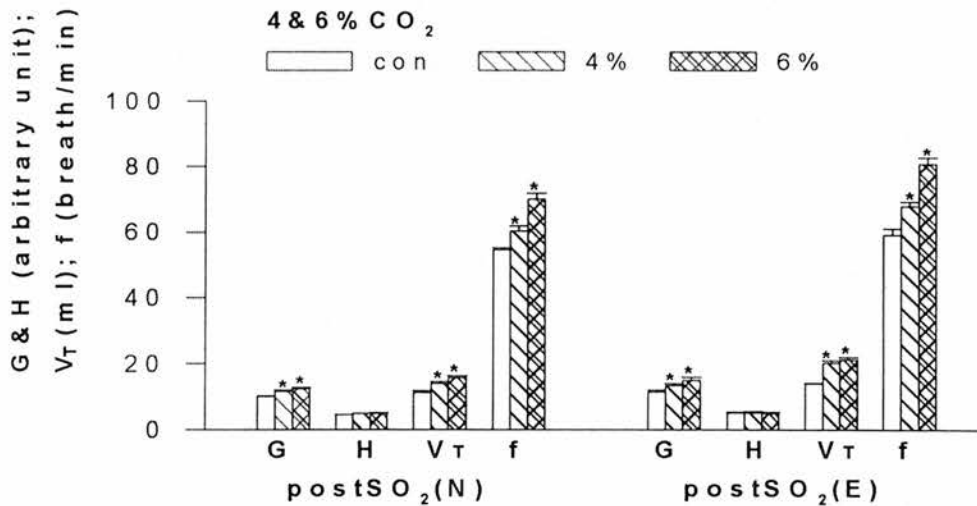


Fig. 3.25 Effect of 4 & 6% CO₂ on pattern of breathing, ventilation and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when SARs were blocked with SO₂ (postSO₂). Expiratory time (t_E, sec), inspiratory time (t_i, sec), tidal volume (V_T, ml), minute ventilation (\dot{V}_I , l/min), frequency of breathing (f, breaths/min), phrenic gradient (G, computer arbitrary units), and phrenic height (H, computer arbitrary units). 4 & 6% CO₂ vs. control breaths (con). Data are mean \pm SEM. * p \leq 0.05, ** p \leq 0.01 & *** p \leq 0.001.

Comparison of the responses in normal and emphysematous rabbits

4% CO₂ inhalation changed the following variables: t_E , t_I , f , Phrenic G & H in both emphysematous and normal rabbits to a similar degree (Table 3.29, Fig. 3.26A&B). However, V_T and therefore \dot{V}_I were increased more in emphysematous rabbits. 6% CO₂ inhalation decreased t_I more in emphysematous as compared to normal rabbits. However t_E was decreased and f increased to a similar degree in both emphysematous and normal rabbits. 6% CO₂ inhalation increased V_T and thus \dot{V}_I more in emphysematous rabbits. 6% CO₂ inhalation increased phrenic G more in emphysematous than in normal animals, however, phrenic H was not affected in both emphysematous and normal rabbits (Table 3.29, Fig. 3.26A&B).

Table 3.29 Comparison of pattern of breathing, ventilation and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to 4 & 6% CO₂, when SARs were blocked with SO₂ (postSO₂). 4 & 6% CO₂ were calculated as percentage change from control breath. Inspiratory time (ti), expiratory time (te), frequency of breathing (f), tidal volume (VT), minute ventilation (\dot{V}_I), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

4 & 6% CO ₂	PostSO ₂	4% CO ₂ % change	6% CO ₂ % change
N(n=25)	te (N)	-8.4±4.7	-27.57±2.4
E(n=35)	te (E)	-13.77±1.7	-27.26±2.3
	ti (N)	-6.67±2.5	-17±2.1
	ti (E)	-12.27±1.5	-24.4±2.2 **
	f (N)	10.89±3.4	30.9±3.3
	f (E)	15.9±1.7	39.16±4.5
	VT (N)	25.4±2.7	36.73±2.9
	VT (E)	46.4±3.5 ***	54.7±3.8 **
	\dot{V}_I (N)	39.16±5.3	78.65±5.4
	\dot{V}_I (E)	69.17±4.1 ***	112.8±6.5 ***
	G (N)	15.4±1.7	24.4±2.3
	G (E)	19.13±1.5	33.92±1.5 *
	H (N)	7.3±3	4.3±4.2
	H (E)	3.17±2.65	-3.1±3.2

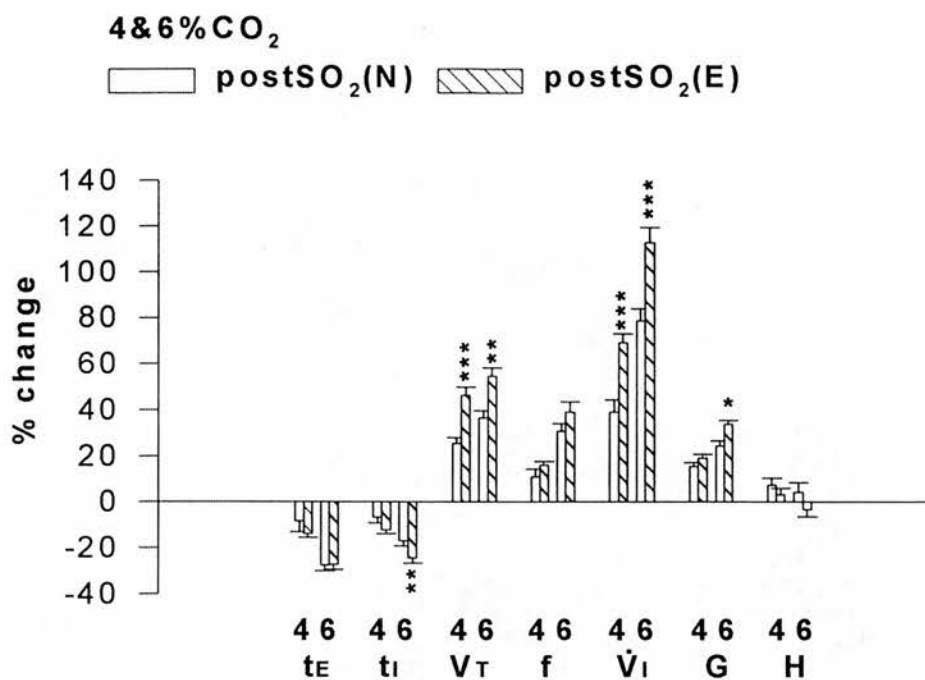


Fig. 3.26 Comparison of pattern of breathing, ventilation and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to 4 & 6% CO₂, when SARs were blocked with SO₂ (postSO₂). 4 & 6% CO₂ were calculated as percentage change from control breath. Inspiratory time (t_I), expiratory time (t_E), frequency of breathing (f), tidal volume (V_T), minute ventilation (V̇_I), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

3.3.5.3 EFFECT OF 4 & 6 % CO₂ ON PATTERN OF BREATHING, VENTILATION AND PHRENIC ACTIVITY AFTER BILATERAL VAGOTOMY

Effect of CO₂ in normal and emphysematous rabbits

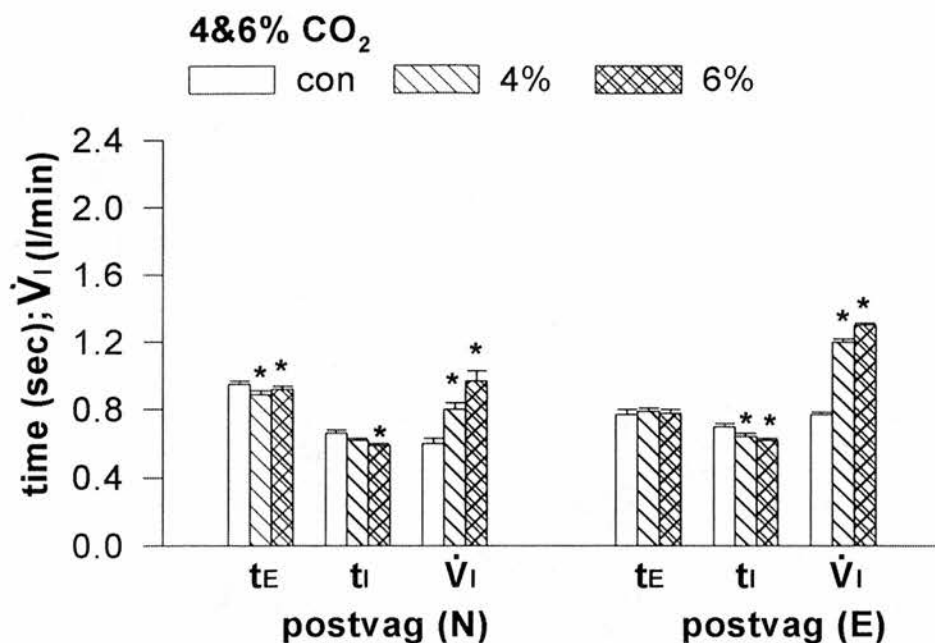
The increase in phrenic H in response to 4 & 6% CO₂ inhalation was only significant in emphysematous rabbits (Table 3.30, Fig. 3.27A&B). In normal and emphysematous rabbits 4 & 6% CO₂ inhalation increased V_T, thus \dot{V}_I was increased. Inhalation of 4 & 6% CO₂ increased phrenic G in both normal and emphysematous rabbits.

In response to 4% CO₂ normal rabbits increased their breathing frequency (f) only through a decrease in t_E, whereas emphysematous rabbits increased f only through a decrease in t_I. After 6% CO₂ normal rabbits increased f through reduction in both t_E and t_I, whereas emphysematous rabbits increased respiratory frequency only through a decrease in t_I (Table 3.30, Fig. 3.27A&B).

Table 3.30 Effect of 4 & 6% CO₂ on pattern of breathing, ventilation and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when all pulmonary receptors were removed by bilateral vagotomy (postvagotomy). Expiratory time (t_E, sec), inspiratory time (t_I, sec), tidal volume (V_T, ml), minute ventilation (\dot{V}_I , l/min), frequency of breathing (f, breaths/min), phrenic gradient (G, computer arbitrary units), and phrenic height (H, computer arbitrary units). 4 & 6% CO₂ vs. control breaths (con). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

4 & 6% CO ₂	Postvagotomy	con	4% CO ₂	6% CO ₂
N(n=20)	t _E	0.95±0.02	0.89±0.02 *	0.92±0.02 *
	t _I	0.66±0.02	0.62±0.01	0.59±0.01 *
	f	37.2±0.35	40±0.47 *	39.86±0.57 *
	V _T	16±0.69	19.32±1 *	24.2±1.3 *
	\dot{V}_I	0.6±0.03	0.8±0.04 *	0.97±0.06 *
	G	7.48±0.19	8.84±0.2 *	9.53±0.25 *
	H	4.16±0.12	4.6±0.16	4.64±0.18
E(n=35)	t _E	0.77±0.03	0.79±0.02	0.78±0.02
	t _I	0.70±0.02	0.64±0.02 *	0.62±0.01 *
	f	41.87±1.16	42.92±1.1 *	43.59±0.99 *
	V _T	18.78±0.66	29.11±0.9 *	30.65±0.71 *
	\dot{V}_I	0.77±0.02	1.2±0.02 *	1.3±0.01 *
	G	7.35±0.49	9.57±0.63 *	10.57±0.63 *
	H	4.39±0.26	5.14±0.29 *	5.42±0.31 *

A



B

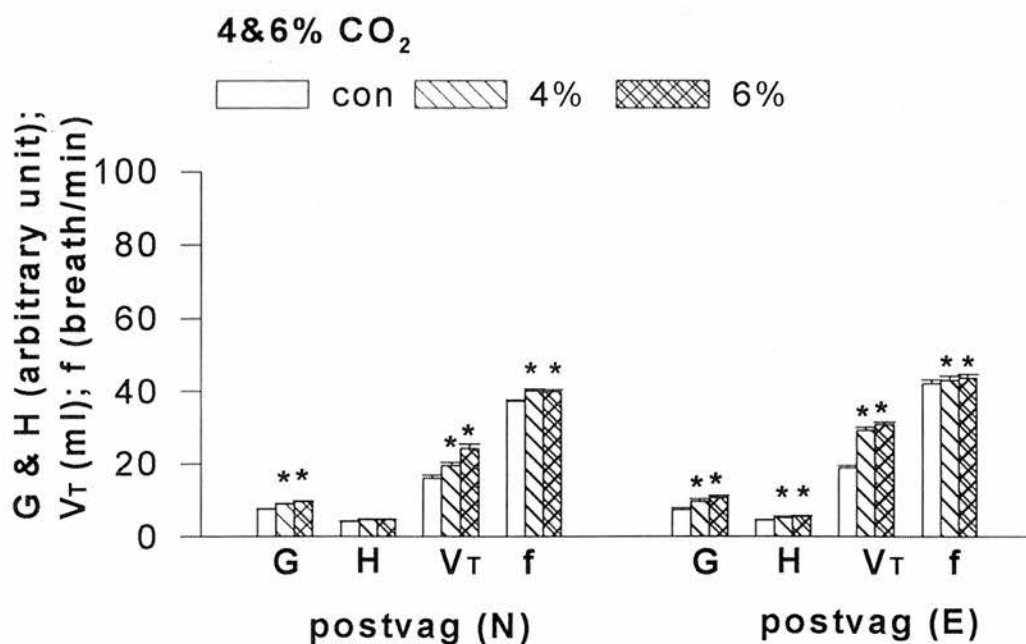


Fig. 3.27 Effect of 4 & 6% CO₂ on pattern of breathing, ventilation and phrenic activity of normal (N) and emphysematous (E) Dutch rabbits, when all pulmonary receptors were removed by bilateral vagotomy (postvagotomy). Expiratory time (tE, sec), inspiratory time (tI, sec), tidal volume (V_T, ml), minute ventilation (\dot{V}_I , l/min), frequency of breathing (f, breaths/min), phrenic gradient (G, computer arbitrary units), and phrenic height (H, computer arbitrary units). 4 & 6% CO₂ vs. control breaths (con). Data are mean \pm SEM. * p \leq 0.05, ** p \leq 0.01 & *** p \leq 0.001.

Comparison of the responses in normal and emphysematous rabbits

In response to inhalation of 4 & 6% CO₂, normal rabbits were able to decrease their t_E , however emphysematous rabbits were not, a difference that was statistically significant (Table 3.31, Fig. 3.28). 4 & 6% CO₂ inhalation also decreased t_I in both emphysematous and normal rabbits to a similar degree. 4% CO₂ inhalation increased f more in normal rabbits, however 6 % CO₂ increased f in emphysematous and normal rabbits to a similar degree. 4 & 6% CO₂ inhalation increased V_T , and thus \dot{V}_I to a greater degree in emphysematous rabbits. They increased phrenic G & H more in emphysematous than normal rabbits; however, the increase in phrenic H did not reach significance (4%: $p=0.06$; 6%: $p=0.08$) (Table 3.31, Fig. 3.28).

Table 3.31 Comparison of pattern of breathing, ventilation and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to inhalation of 4 & 6% CO₂, when all pulmonary receptors were removed by bilateral vagotomy (postvagotomy). 4 & 6% CO₂ were calculated as percentage change from control breath. Inspiratory time (ti), expiratory time (te), frequency of breathing (f), tidal volume (VT), minute ventilation (\dot{V}_I), phrenic gradient (G), phrenic height (H). Data are mean \pm SEM. * p \leq 0.05, ** p \leq 0.01 & *** p \leq 0.001.

4 & 6% CO ₂	Postvagotomy	4% CO ₂ % change	6% CO ₂ % change
N(n=20)	te (N)	-6.61 \pm 0.98	-3.3 \pm 1.04
E(n=35)	te (E)	2.93\pm1.59 ***	3\pm1.54 **
	ti (N)	-6.29 \pm 2.19	-10.06 \pm 2.25
	ti (E)	-7.45\pm1.7	-10.98\pm1.54
	f (N)	7.56 \pm 1.24	7.1 \pm 1.17
	f (E)	3\pm1.14 *	4.78\pm1.15
	VT (N)	21.04 \pm 4.7	51.44 \pm 6.28
	VT (E)	57.58\pm4.1 ***	66.26\pm3.66 **
	\dot{V}_I (N)	29.73 \pm 4.5	61.88 \pm 6.38
	\dot{V}_I (E)	61.43\pm3.53 ***	73.29\pm2.96 *
	G (N)	18.98 \pm 3.13	27.95 \pm 3.2
	G (E)	31.2\pm1.2 ***	45.61\pm2.08 ***
	H (N)	12.21 \pm 5.23	13.13 \pm 5.43
	H (E)	20.22\pm3.36	26.39\pm3.82

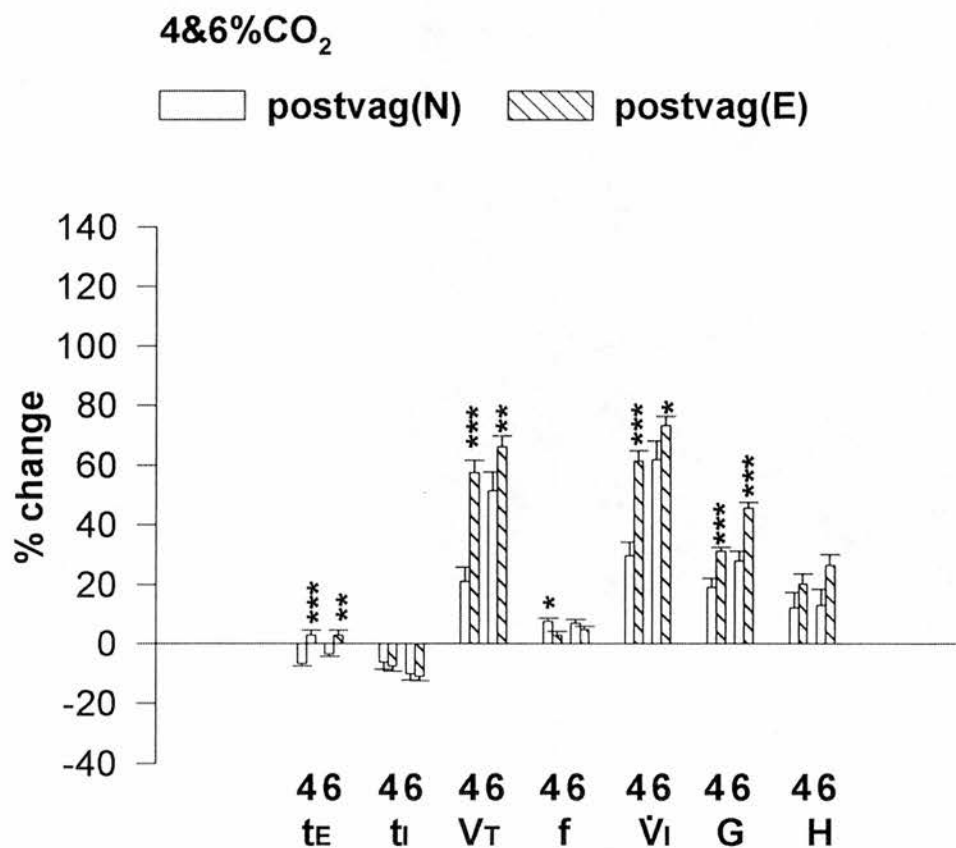


Fig. 3.28 Comparison of pattern of breathing, ventilation and phrenic activity between normal (N) and emphysematous (E) Dutch rabbits in their response to 4 & 6% CO₂, when all pulmonary receptors were removed by bilateral vagotomy (postvagotomy). 4 & 6% CO₂ were calculated as percentage change from control breath. Inspiratory time (ti), expiratory time (tE), frequency of breathing (f), tidal volume (VT), minute ventilation (VI), phrenic gradient (G), phrenic height (H). Data are mean±SEM. * p≤0.05, ** p≤0.01 & *** p≤0.001.

Chapter 4

4. DISCUSSION

4.1 CHARACTERISTICS OF RABBIT EMPHYSEMA

In any study of a disease model, it is important that the model accurately reflects those aspects of the disease to be studied. Snider *et al.* (1986) pointed out that “the usefulness of an experimental model should be judged on how well it answers the specific questions it is being used to answer, rather than how well it mimics human disease”. The model used in the present study, using rabbits treated with elastase and rats treated with papain, is intended to change the architecture of the lungs in the same way as emphysema does in humans.

Objective assessment of airspace diameter: mean linear intercept (Lm) showed that these were increased in the present model. Lm of the emphysematous rat was 34% greater than normal, and that of rabbit was 37% greater than normal. Results obtained in the rabbits showed that both lungs and all lobes were affected. This suggests that emphysema had successfully been produced in both of these models. Similar increases in Lm were found in other models of emphysema in a variety of animal species (Lucey, O'Brien, Pereira & Snider, 1980; Damon, Mauderly & Jones, 1982). In examining the lungs histologically, normal lungs appeared to have a finely interlacing alveolar structure with no evidence of emphysema, whilst emphysematous lungs appeared to show areas of emphysematous lesion interspersed with areas of normal lung, which is consistent with the findings of others (Kilburn, Dowell & Pratt, 1971). In addition, in the present investigation, an experienced pulmonary pathologist Dr. D Lamb (appendix 2), from The Pathology Department, Medical School, Edinburgh University, confirmed the presence of emphysema subjectively by contrasting two slides from normal rabbits and two from emphysematous rabbits, which were chosen randomly from the whole collection of slides (Appendix 2).

Lung compliance, which is a measure of elastic recoil, is also affected in emphysema (Panettieri, 1995). Due to destruction of lung tissue and loss of elastic fibres emphysematous lungs have a higher static compliance, i.e. a given volume is

produced by a smaller pressure change. Measurement of static compliance of the normal and emphysematous animals was measured in situ. The compliance of normal animals found in the present study (rabbits: 4.5 ± 1.5 ml/cm H₂O; rats: 0.24 ± 0.02 ml/cm H₂O) is similar to that reported by Crosfill & Widdicombe (1961) (rabbits: 3.5-10.8 ml/cm H₂O; rats: 0.22-0.52 ml/cm H₂O). The present study showed that emphysema increased static compliance in both rats and rabbits, and that is in agreement with the finding that emphysema induced with either papain or pancreatic elastase in a variety of animal species produce an increase in static compliance (Snider, Lucey & Stone, 1986). This further confirms that emphysema had successfully been induced in both rats and rabbits.

In emphysema, changes in blood gas tension may occur. In the present study measurement of blood gases were performed in 7 normal and 7 emphysematous rabbits. Results showed that the P_{aCO_2} in both normal and emphysematous rabbits was within the normal range, whilst some emphysematous rabbits were hypoxic and so too were some normal rabbits.

The values of arterial blood gas tensions of emphysematous rabbits obtained in the present study are consistent with the findings of others that arterial blood gas tension in emphysematous animals and humans showed normal values or hypoxemia without CO₂ retention (Snider *et al.*, 1986). Ventilation-perfusion mismatch seems to be the most likely explanation for the hypoxemia. Alteration of diffusion function related to decreased internal surface area of the lung (Goldstein, Karkinsky & Snider, 1977) could also have been a factor. However Lucey, Snider & Javaheri (1980) found that emphysematous hamsters were able to increase their P_{aO_2} during exercise which is evidence against a reduction in diffusing capacity in those animals.

The cause of hypoxemia in normal rabbits is however unclear. O'Brien, Lucey & Snider (1979) found that values for the P_{aO_2} of normal, awake, unrestrained hamsters were low compared to those obtained in humans and dogs showing a species difference. Unfortunately, similar studies have not been performed in rabbits. Aeschbacher & Webb (1993) reported the presence of hypoxemia in normal rabbits anaesthetized with propofol; however, the necropsy findings did not suggest that pulmonary congestion and atelectasis were important factors. Korner, Uther & White (1968) were unable to maintain normal arterial blood gases tensions during

spontaneous ventilation of rabbits positioned on their back, and a supported crouching and sternal position was used for their experiments. In the present experiments, the use of the sternal position was impossible because of the difficulty it would impose on nerve dissection and pattern of breathing measurements. Inflation pressure used in this study would act to overcome pulmonary atelectasis and 100% oxygen was given to all rabbits to obliterate any hypoxic drive to breathe. Cardiovascular causes of hypoxemia were excluded because mean arterial blood pressure was normal in both normal and emphysematous rabbits. The in vitro lung volume of rabbits was not changed by emphysema, a finding consistent with that of Damon, Mauderly & Jones (1982) who found that lung volume was not changed by a dose of elastase less than 800IU/kg. In conclusion emphysema was successfully induced in rats and rabbits. The models produced had functional, morphological and morphometric changes consistent with models produced in a variety of animal species and are consistent with human emphysema.

4.2 CONSCIOUS PATTERN OF BREATHING

Studies using the component of variance technique showed that a smaller number of Dutch rabbits compared to New Zealand White rabbits were required to detect a 10% change in conscious pattern of breathing.

Rabbits

One month after the induction of emphysema with porcine pancreatic elastase, emphysematous conscious rabbits showed no change in their pattern of breathing (t_E , t_I & V_T ; Results: Table 3.14). The absence of detectable change in the conscious rabbit pattern of breathing could be due to the requirement for a much larger number of rabbits in the conscious state to detect the same level of change due to higher variability in the conscious than anaesthetised pattern.

Unfortunately it was not possible to do more experiments, because of the rabbits' uncooperativeness. The increase in frequency of the normal anaesthetised intubated rabbits compared to normal conscious rabbits may have been due to the endotracheal intubation bypassing the upper airway resistance and/or the effects of anaesthetic (Results: Table 3.14 & Table 3.16).

Rats

Two weeks after the induction of emphysema with papain, measurement using the barometric method showed that emphysematous conscious rats had longer t_E and larger V_T (Results: Table 3.14). Mansoor, Hyde & Schelegle (1997) used the same substance to induce emphysema and the same barometric method for assessing the conscious pattern of breathing in rats, two weeks after induction of emphysema. However, they found a significant increase in t_I , with no significant change in t_E ; V_T was not measured. One of the differences in protocol was that Mansoor & colleagues (1997) measured the pattern of breathing on only one day, before and after the induction of emphysema. In addition they did not state the number of breaths measured on each occasion. The protocol used in the present study i.e. the use of multiple breaths and measurement of the pattern of breathing on four consecutive days and the animals serving as their own control is probably more efficient in detecting differences as indicated by component of variance (Result: Tables 3.9, 3.12). Lucey, Snider & Javaheri (1982) also used the barometric method to study pattern of breathing of hamsters rendered emphysematous by intratracheal instillation of porcine pancreatic elastase. The pattern of breathing was measured 1 month, 5 months and 13 months after the induction of emphysema. They found that after one month, emphysematous hamsters had no change in their pattern of breathing. However by 5 months, they had longer t_I & t_E , lower f & larger V_T , and by 13 months they had larger V_T & longer t_I . The mean linear intercept was found to be greater after 1, 5 and 13 months. They attributed their findings to factors other than emphysema, such as changes in the temperature of the expired air. In contrast to the present protocol, Lucey *et al.* (1982) only used five consecutive breaths to measure the pattern of breathing and the pattern was only measured on one occasion. However, their results could indicate the importance of ageing of the emphysematous lesion in hamsters. Despite the differences in protocol, the results obtained in the present study showing increased V_T and t_E agree with the results obtained by Lucey *et al.* (1982) in hamsters 5 months after the induction of emphysema.

The conscious pattern of breathing of emphysematous horses was measured using a pneumotachograph attached to a face mask (Gillespie, Tyler & Eberly, 1966).

The results showed that emphysematous horses had lower V_T , a shorter t_I , and their t_E & f were unchanged. The differences from the results of the present study could have been due to the fact that unlike the emphysema induced in the present rat model, emphysema in horses had occurred naturally, so it was probably associated with other medical pathological conditions. Seven out of nine horses' lungs had emphysema as the most pronounced disease, however lungs from one had severe diffuse infectious bronchoadenitis, and that from another had areas of bronchitis and bronchiolitis. Lungs of two diseased horses were not examined (Gillespie *et al.*, 1966).

The conscious pattern of breathing of human subjects with COPD was measured using a respiratory inductive plethysmograph which does not require a mouth piece since this is known to change pattern of breathing by increasing V_T and reducing f (Gilbert *et al.*, 1972; Hirsch & Bishop, 1982). Tobin, Chadha, Jenouri, Birch, Gazeroglu & Sackner (1983) studied pattern of breathing of eucapnic COPD subjects and concluded that they had larger V_T , shorter t_I & higher f than normal matched subjects. Loveridge, West, Anthonisen & Kryger (1984) using a modification of the same method to analyse timing components, found that COPD subjects had shorter t_I & t_E , higher f , but V_T was unchanged. They attributed the difference in their results from those of Tobin *et al.* (1983) to a difference in posture of the subjects. Tobin *et al.* (1983) subjects were studied in the supine position whilst those of Loveridge *et al.* (1984) were studied whilst seated in a dental chair. Loveridge *et al.* (1984) found that their normal subjects had larger V_T , \dot{V}_I and shorter t_I than Tobin's normal subjects. The fact that their subjects had COPD and not pure emphysema as in the present study could have been responsible for the differences in their findings compared to these found in this study. Despite this Tobin *et al.*, 1983) found an increase in V_T , in agreement with that found in the present study.

4.3 ACUTE EXPERIMENTS

4.3.1 EFFECTS OF SO₂ AND VAGOTOMY ON PATTERN OF BREATHING, VENTILATION AND PHRENIC ACTIVITY

Reasons for the use of SO₂ and vagotomy

The fact that the pattern of both types of pulmonary receptor (slowly adapting pulmonary stretch receptors {SARs} & rapidly adapting receptors {RARs}) discharge is changed in the model of emphysema (Davies & Pirie, 1995) creates difficulty in interpretation of results. To elucidate the role of each type of pulmonary afferent in the changed pattern of breathing and the drive to breathe in the rabbit model of emphysema investigated in the present study, SARs were blocked by sulphur dioxide (SO₂) which is known to selectively block SARs and to leave other pulmonary receptors intact (Davies, Dixon, Callanan, Huszcuk, Widdicombe & Wise, 1978). In the present study, complete blockade of SARs was verified by the absence of the Hering Breuer inflation reflex (HB). Since, almost all pulmonary afferents are carried in the vagus nerves, bilateral vagotomy provided a basic, centrally generated respiratory pattern from which the effects of vagal afferents were removed.

Effect of blocking SARs with SO₂

For normal rabbits, in the present study, the increase in t_I and V_T after blockade of SARs with SO₂, are in accordance with the findings of Davies *et al.* (1978). It has been suggested that the inputs from SARs cause the termination of inspiration through an off-switch mechanism (Clark & von Euler, 1972). Hence the removal of these inputs causes an increase in t_I and V_T .

The increase in t_E found in the present study, after blocking SARs with SO₂, is in agreement with Mortola, Fisher & Sant'Ambrogio (1984) who also used urethane as an anaesthetic. However, Citterio, Piccoli & Agostoni (1985) who used a combination of urethane and Nembutal as an anaesthetic, found that t_E did not change significantly, but Davies, Dixon, Callana, Huszczuk, Widdicombe & Wise (1978) who used Nembutal, found a decrease in t_E . Thus, there is a clear indication that the effect on t_E is related to the anaesthetic used. It was proposed that the shortening of t_E after SARs block with SO₂ is due to the removal of inputs from

SARs acting to inhibit the central inspiratory activity during expiration (Davies *et al.* 1978; Knox 1973, 1979; Cohen & Feldman, 1977). Citterio *et al.* (1985), who found no change in t_E , proposed two other mechanisms. Firstly, the increase in t_I following SO_2 block should increase t_E indirectly through the central drag of t_I on t_E (Cohen, 1969; Clark & von Euler, 1972). This was supported by their finding that t_E decreased when the increase in t_I was small, but it increased when the increase of the t_I was large. Secondly, Citterio *et al.* (1985) proposed that the removal of the inspiratory facilitatory input from SARs operating at FRC should increase t_E . Mortola, Fisher & Sant'Ambrogio (1984) did not propose any detailed mechanism for the change in breathing pattern which they observed in their study apart from the effects of different anaesthesia.

Because the observed changes t_E appear to be related to anaesthetic used, it was decided to compare in detail the results from the different studies (Table 4.1). It can be seen that before the application of SO_2 , the initial values of t_E are very much shorter when urethane is used as an anaesthetic than with Nembutal, in fact t_E is approximately twice as long when Nembutal is used. The t_I is also slightly shorter in the presence of urethane. It may be at lower t_E and t_I in the presence of urethane, the facilitatory inputs from SARs are operative, and the removal of these inputs by SO_2 block would cause an increase in t_E as proposed by Citterio *et al.* (1985). In support of this hypothesis is the finding of the present study that SARs block with SO_2 reduced phrenic G as an indication to the removal of the facilitatory input. However, Davies *et al.*, (1978) proposed that the removal SARs input would act to decrease t_E . Thus, a more clear understanding of the effects of anaesthesia on respiratory control is required to elucidate whether SARs act to lengthen t_E (Davies *et al.*, 1978) or to shorten t_E (Citterio *et al.*, 1985).

Table 4.1 Effect of SO₂ and vagotomy on pattern of breathing in the present study compared to other studies. tE: expiratory time, tI: inspiratory time, U: urethane, N: Nembutal.
 postSO2 (Δ %) was calculated as % change from preSO2 and postvagotomy was calculated as % change from postSO2.

		Pre SO2	Pre SO2	Pre SO2	Post SO2	Δ %	Post SO2	Δ %	Post vagotomy	Δ %	Post vagotomy	Δ %
	Anaesthetic	tE (sec)	tI (sec)	tE	tI	tE	tI	tE	tI	tE	tI	tI
Present study	U	0.49	0.39	0.71	0.59	45	51	0.95	0.66	34	12	
Mortola, 1984	U	0.33	0.33	0.49	0.52	48	58	0.69	0.68	40	31	
Citterio, 1985	U+N	0.96	0.45	0.83	0.67	-14	49					
Davies, 1978	N	0.79	0.44	0.6	0.8	-24	82	1.05	1.2	75	50	
Present study	none	0.67	0.54									

In the present study, SO_2 decreased phrenic slope significantly and there was also a significant increase in t_i , therefore phrenic height was not changed statistically. Davies *et al.* (1978) reported no significant increase in phrenic height. They also reported no change in phrenic slope (G) which is in contrast to the reduction found in the present study. The reduction of G, in the present study, is in agreement with the finding that SARs provide a facilitatory input during eupenic breathing (Bartoli, Cross, Guz, Huszczuk & Jefferies, 1975; Cross, Jones & Guz, 1980; DiMarco, von Euler, Romaniuk & Yamamoto, 1981; Citterio & Agostoni, 1983; Hwang & St. John, 1993). Citterio *et al.* (1985) also found changes in respiratory drive when they measured the diaphragmatic EMG after SO_2 administration. They found that during the first part of inspiration the rate of rise of diaphragmatic EMG under SO_2 block was smaller than control, whilst in the latter part of inspiration the reverse was true. It is not clear whether there was an overall decrease, as the rate of rise of diaphragmatic EMG for the whole of inspiration was not measured. The idea of SARs providing facilitatory input is also supported by Pisarri, Yu, Coleridge & Coleridge (1986) who found that the mean rate of inspiratory flow (V_T / t_i), which provides an index of inspiratory drive, decreased slightly when the vagus nerve was cooled to 7°C , a temperature known to block conduction in large (SARs) myelinated fibers. The decrease was maximal when the temperature reached 3°C at which point, all pulmonary receptors are blocked.

Effect of bilateral vagotomy

For normal rabbits, in the present study, elongation of t_E , by vagotomy is in agreement with results obtained in several studies (Davies *et al.*, 1978; Guz & Trenchard, 1971; Russell, Raybould & Trenchard, 1984; Pisarri, Yu, Coleridge & Coleridge, 1986). It can be attributed to the loss of input of one or other remaining pulmonary receptors, RARs and C-fibres. In the present study, no attempt has been made to separate the reflex effects of these two types of receptors.

Some researchers (Fishman, Phillipson & Nadel, 1973; D'Angelo, 1980) consider RARs as the major factor limiting t_E during quiet breathing under normal conditions. Others (Guz & Trenchard, 1971; Russell, Raybould & Trenchard, 1984) concluded that background activity in nonmyelinated fibers influence breathing

pattern only in diseased conditions. However, Miserocchi, Trippenbach, Mazzarelli, Jaspar & Hazucha (1978) and Pisarri, Yu, Coleridge & Coleridge (1986) believe that C-fibers are more important. They suggested that in eupnoea low frequency, background activity in pulmonary afferent C-fibers shortens t_E and that the lengthening of t_E after vagotomy is due to the loss of their input.

The further lengthening of t_I after vagotomy, in the present study, has been observed in other forms of vagal block (Karczewski & Widdicombe, 1969; Nadel, Phillipson, Fishman & Hickey, 1973; Phillipson, Fishman, Hickey & Nadel, 1973). This prolongation is difficult to ascribe to RAR, since their stimulation only causes augmentation of t_I (Davies & Roumy, 1986). Stimulation of C-fibers is known to produce a tachypnic response with the shortening of t_E and t_I (Coleridge & Coleridge, 1984). The more likely explanation for this prolongation is that there may be a central linking relationship between t_E and t_I , so that when t_E is prolonged it lengthens t_I as suggested by Davies & Roumy (1986).

For normal rabbits, in the present study after vagotomy there was no further decrease in phrenic slope (G), but an increase was observed in phrenic height (H), which is in agreement with the finding of Davies *et al.* (1978). This increase in H was associated with an increase in V_T and t_I .

This study showed that blockade of SARs with SO_2 increased t_I and V_T showing the importance of inputs from SARs to act as an off switch. The decrease in phrenic G confirms others finding that SARs provide facilitatory inputs before acting as an off switch (Bartoli *et al.*, 1975; Cross *et al.*, 1980; DiMarco *et al.*, 1981; Citterio & Agostoni, 1983; Hwang & St. John, 1993). Further lengthening of t_E after bilateral vagotomy, indicates that the other remaining pulmonary receptors i.e. RARs and C-fibres, act to shorten it.

4.3.2 COMPARING NORMAL AND EMPHYSEMATOUS DUTCH RABBITS IN THE CONTROL STATE AND IN THEIR RESPONSE TO SO₂ AND BILATERAL VAGOTOMY

The results obtained in the present study (Results: Table 3.16 & Fig. 3.9) show that emphysematous rabbits, compared to 25 normal rabbits, have greater V_T , \dot{V}_I and a longer t_E . The results also show that phrenic slope and height are 28% and 31% respectively greater in emphysematous rabbits compared to normal, however the bigger standard deviation of the results for emphysematous rabbits prevented this finding reaching statistical significance (Results: Table 3.16 & Fig. 3.9).

These findings are in agreement with that obtained by Damon, Mauderly & Jones (1982) who studied the effect of emphysema in pathogen-free rats anaesthetised with halothane, the rate of administration of which was adjusted to keep breathing frequency constant; the pattern of breathing was measured 7-144 days after induction of emphysema with porcine pancreatic elastase (PPE). These workers also found that emphysematous rats have a greater V_T & \dot{V}_I .

In contrast, Delpierre, Fornaris & Payan (1985), who studied the effects of emphysema in rabbits anaesthetised with pentobarbitone, where the pattern of breathing was measured seven months after induction of emphysema with PPE found no difference between the treated and untreated groups. The lack of differences observed in this study could have been due to the smaller number of rabbits used (7 treated vs. 8 untreated) than in the present study where 35 treated vs. 25 normal animals were used. In support of this suggestion is the fact that t_E before phenyldiguanide (PDG), which was used to assess the pulmonary chemoreflex mediated by C-fibres, in their study was increased 27% in treated animals, however the big coefficient of variation (13%) made this increase statistically insignificant, indicating the importance of having a larger number of rabbits for study. The increase in t_E in the present study, however, was only 16% but there was a very small coefficient of variation (4%). However between two different doses of PDG (20 $\mu\text{g/kg}$ & 30 $\mu\text{g/kg}$) Delpierre *et al.*, (1985) found that emphysematous rabbits had a lower V_T and longer t_I .

Mansoor, Hyde & Schelegle (1997), who studied the effect of emphysema in rats anaesthetised with a combination of urethane and chloralose, where the pattern

of breathing was measured 14-17 days after induction of emphysema with PPE, found that pattern of breathing was not different in emphysematous compared to normal rats. The reasons for this discrepancy may be due to a species difference. However, the fact that they compared elastase treated animals to a group of animals consisting of a combined group of absolute control and saline treated may have contributed to the lack of differences. The absolute control and saline treated animals were shown to differ in their lung surface area. The pattern of breathing of the two groups was said to be similar, however the data were not presented in the paper.

In the present investigation, emphysematous rabbits were found to have a longer t_E , and a larger V_T and \dot{V}_I . Pirie (1997) found that SARs have greater activity in emphysematous rats. Activity of SARs is known to inhibit inspiratory activity during expiration (Knox, 1973, 1979; Cohen & Feldman, 1977). Thus it is possible that the longer t_E found in emphysematous rabbits in the present study could be due to an increase in SARs activity. In addition, Pirie (1997) also found that the RARs are more active in rats. RARs are known to be excitatory to breathing. Therefore, if a similar increase in RARs activity occurred in rabbits this could account for the increase in respiratory drive. In the present study, no attempt has been made to separate the reflex effect of RARs and C-fibres. However, previous studies in animal models of pulmonary emphysema showed that reflex activity of C-fibres was not changed in emphysematous animals (Delpierre *et al.*, 1985; Mansoor *et al.*, 1997). It is known that transpulmonary pressure is a more representative stimulus than lung volume for SARs activation (Knowlton & Larrabee, 1946). An increase in lung static compliance means a given lung volume is achieved by smaller pressure changes and tension in the lung, and therefore increased static compliance is expected to reduce SARs discharge. However, Sant'Ambrgio, Sant'Ambrgio & Fisher (1988) found, in normal dogs that 22% of intrapulmonary SARs are activated by the increased compliance which was brought about by hyperinflation, 61% reduced their activity and 17% had no change in their activity. Increases in compliance brought about by hyperinflation, is different from the increased compliance of the emphysematous lungs, where there is loss of elastic recoil of the lung due to destruction of the lung parenchyma. It is expected that the dissociation of transpulmonary pressure and SARs activity will be exaggerated in the higher lung static compliance associated

with emphysema, and those receptors which showed greater activity may overpower the ones with reduced activity.

Emphysematous vs. normal animals in their response to SO₂

Removal of SARs activity by SO₂ increased V_T in both normal and emphysematous rabbits. It increased t_E in normal animals whilst that of emphysematous rabbits remained unchanged. This difference was statistically significant (Results: Table 3.17 & Fig. 3.10A&B). The difference in the control value (pre SO₂) could affect the way that SO₂ administration changes t_E. A long t_E as seen in animals anaesthetised with Nembutal (0.79sec) was found to be shortened by SO₂, however a short t_E (0.49) observed in animals anaesthetised with urethane was found to be lengthened. Emphysematous rabbits under urethane anaesthesia, had a t_E value (0.57) intermediate between that of urethane and Nembutal. It might be that under these conditions SO₂ causes neither lengthening nor shortening of t_E. Normal rabbits under urethane anaesthesia had a short t_E (0.49) that was lengthened by SO₂. Anaesthetics also differ in the way in which they affect the t_E / t_I ratio. For example, in the present study, urethane kept the ratio similar to that of the conscious pattern, however Nembutal administration resulted in a larger ratio (Table 4.1).

The differences in t_E changes in response to SO₂ administration between normal and emphysematous rabbits may also be interpreted as a difference in receptor activity. Therefore, it is probable that in emphysematous rabbits, t_E was largely determined by RAR/C-fibres that act to shorten t_E (Davies & Roumy, 1986; Pisarri, Coleridge & Coleridge, 1986). The t_I of emphysematous rabbits was not increased as much as that observed in normal animals on SO₂ block; this might be due the fact their t_E was not increased which affected the value of t_I fixed by the central t_I - t_E relationship (Results: Table 3.17 & Fig. 3.10A&B). In the present study, SO₂ increased phrenic G in emphysematous animals, whilst reducing G in normal animals. It also greatly increased phrenic H in emphysematous rabbits, whilst that of normal rabbits remained unchanged (Result: Table 3.17). These differences in phrenic activity indicate that emphysematous rabbits have a larger vagally mediated drive to breathe initiated by RARs (Results: Table 3.17 & Fig. 3.10A&B).

Emphysematous vs. normal animals in their response to bilateral vagotomy

In the present study, bilateral vagotomy increased t_E & t_I more in emphysematous animals (Table 3.17 & Fig. 3.10). In contrast Mansoor *et al.* (1997), who studied the effect of vagotomy in emphysematous rats, concluded that there was no difference between the control and emphysematous pattern of breathing (V_T , f , t_E & t_I) after vagotomy. However, they did not state how the comparison was made, and the data was not presented. In the present study, comparison was made between normal and emphysematous rabbits, when the postvagotomy effects were calculated as a percentage change from the results of post SO_2 . The greater reduction of f & \dot{V}_I in emphysematous rabbits, in the present investigation, followed the greater increase in t_E and t_I . Bilateral vagotomy did not change Phrenic G any further in normal, whilst G of emphysematous rabbits was greatly reduced. Bilateral vagotomy slightly increased phrenic height (H) in normal, whilst H of emphysematous rabbits was not significantly changed, however the difference between groups was not statistically significant (Results: Table 3.17, Fig. 3.10B). These results of phrenic activity after bilateral vagotomy may lend a further support to the earlier conclusion, that there was a larger vagally mediated drive to breathe emanating from RARs/C-fibres in emphysematous rabbits after blockade of SARs. It is probable that removal of this larger drive to breathe, by vagotomy, had led to the greater reduction in phrenic G of emphysematous rabbits.

The greater increase of t_E in emphysematous rabbits by bilateral vagotomy could be due to the removal of input from RAR/ C-fibres, which was shown to act to shorten t_E after blocking SARs by SO_2 . The greater increase of t_I in emphysematous rabbits was probably the result of a central effect linking t_E - t_I .

Two previous studies have investigated the reflex effect of C-fibres in emphysematous compared to normal animals. Delpierre *et al.* (1985) studied changes in the pulmonary chemoreflex (PCR), which is mediated by pulmonary C-fibres. PCR was evoked by an injection into the right atrium of capsaicin. These workers concluded that there was no difference in the PCR between normal and emphysematous rabbits. Similarly Mansoor *et al.* (1997) studied PCR in

emphysematous rats, which was evoked by a right atrial injection of phenyldiguanide. They too concluded that PCR was not changed.

In the present study, the reflex effect of inputs from RARs in normal and emphysematous Dutch rabbits, has been examined by studying the effect of the deflation reflex. However the reflex activity of C-fibres was not investigated.

Blockade of SARs with SO_2 revealed that t_E in emphysematous rabbits is largely determined by RARs/C-fibres, that act to shorten it. This could imply that RARs/C-fibres are more active in emphysematous rabbits. The changes in phrenic activity after SO_2 i.e. the increase of phrenic G in emphysematous rabbits, the reduction of phrenic G in normal animals, and the greater increase of phrenic H in emphysematous rabbits could also indicate the presence of more active RARs/C-fibres in emphysematous rabbits mediating this higher drive. Removal of other pulmonary receptors (RARs/C-fibres) by bilateral vagotomy led to a greater increase in t_E and t_I in emphysematous rabbits than normal animals. This might indicate removal of more active RAR/C-fibres in emphysematous than in normal animals. Also indicative of the greater activity of RAR/C-fibres in emphysematous rabbits is the larger reduction in phrenic G in emphysematous rabbits compared to normal animals after bilateral vagotomy.

4.3.3 THE EFFECT OF INFLATION IN NORMAL AND EMPHYSEMATOUS RABBITS

In the present study, for normal rabbits, before SO_2 administration, inflation with 5 cmH₂O increased t_E of the 1st, 2nd & 5th breath, i.e. producing a Hering-Breuer inflation reflex (HBIR) (Results: Table 3.18A&B, Fig. 3.11A&B). The t_E of the 1st breath was 2 times that of control, following which there was a progressive decrease in HBIR in the 2nd and 5th breath. Davies & Roumy (1986) found the increase in t_E to be 4 times that of control. They also reported the progressive decrease in HBIR in the 2nd and 5th breath. The cause of this progressive decrease in HBIR could be the adaptive property of SARs. Bartlett & St. John (1979) found that the half time of adaptation of these receptors in rabbits is 0.75 ± 0.37 sec, therefore the 1st breath under

inflation will lie within the half time, whilst that of 2nd and 5th breath will be outside the half time of adaptation.

For normal rabbits, the increase in t_E produced by inflation with 10 cm H₂O was 10 times that of the control. Similarly Davies *et al.* (1978) reported an increase that was 11 times that of the control. In the present study, for normal rabbits, before SO₂ administration, inflation with 5 cm H₂O did not significantly affect t_i . In contrast Davies & Roumy (1986) found an increase in t_i of the 1st breath and a decrease in t_i in those of the 2nd and 5th breaths. However, only 5 rabbits were studied and their data was presented qualitatively without subjection to statistical analysis.

Furthermore, in the present study, inflation with 10 cmH₂O produced an increase in phrenic slope and height in the first inspiratory effort, indicating a higher drive to breathe, which probably was due to asphyxia (Table 3.18 & Fig. 3.11). Asphyxia may have also contributed to the progressive decrease in HBIR in the 2nd and 5th breaths during 5 cm H₂O inflation.

After the influence of the SARs was removed by SO₂ block the HBIR was abolished, and instead inflation produced a shortening of t_E in 1st, 2nd and 5th breaths (Table 3.20A&B, Fig. 3.14A & Fig. 3.15A), which is in agreement with the finding of Davies & Roumy (1982, 1986). After the removal of the predominant effect of SARs activity, which caused the HBIR, it is likely that the stimulation of the RARs by inflation resulted in this decrease in t_E .

For normal rabbits, after SO₂ administration, inflation with 5 cmH₂O increased the t_i of the 1st breath but decreased that of the 2nd and 5th breaths (Table 3.20 & Fig. 3.14), a finding which is in agreement with that of Davies & Roumy (1986). The increase in the 1st breath was attributed to triggering of an augmented breath through RARs. The decrease in t_i of the 2nd and 5th breaths could be due to the decrease in t_E causing a decrease in t_i through a system of t_E - t_i central linking. After SO₂ administration, inflation with both 5 & 10 cmH₂O caused an increase in phrenic slope (Table 3.20 & Fig. 3.14). Since apnoea no longer occurs, asphyxia can not be implicated. It is possible that RARs could be mediating this effect, as inflation is known to stimulate them (Davies & Roumy, 1986).

In the present study, before SO₂ administration, the HBIR tended to be stronger in normal rabbits (N) compared to emphysematous (E) animals. However there was no statistical difference between the two groups. This was due to the large coefficient of variation (+5 cmH₂O, c.v.(N)=50%, c.v.(E)=39%; +10 cmH₂O, c.v.(N)=126%, c.v.(E)=78%) (Table 3.19 & Fig. 3.13). Delpierre *et al.* (1985) who studied the changes in the HBIR in rabbits 7 months after induction of emphysema, found that the strength of the HBIR was greater in emphysematous rabbits, at an inflation volume greater than 30 ml. However, when HBIR was expressed as a function of transpulmonary pressure, no significant difference between groups was obtained. Mansoor *et al.* (1997) studied HBIR in rats 2 weeks after induction of emphysema and found no change in the strength of the HBIR.

In the present study, after SO₂ administration, inflation with 5 cmH₂O shortened t_E of 1st & 2nd breaths more in normal than emphysematous animals. This could be due to emphysematous (E) and normal (N) rabbits starting from different control values of t_E (N: 0.63±0.02; E: 0.52 ±0.02;).

4.3.4 THE EFFECTS OF DEFLATION IN NORMAL AND EMPHYSEMATOUS RABBITS

In the present study, for normal rabbits, deflation the lung with 5 cmH₂O & 10 cm H₂O reduced t_E of the 1st, 2nd and 5th breaths. It increased t_I of the 1st breath, and that of 2nd and 5th were also increased but not significantly. It increased phrenic slope and height of the 1st, 2nd and 5th breaths. These findings are in agreement with these of Davies & Roumy, 1986 and Coleridge & Coleridge, 1986 who suggested that the deflation reflex increases breathing frequency by decreasing t_E and augments respiration by increasing phrenic height (H) and slope (G). Deflation reduces input from SARs and increases that from RARs (Knowlton & Larrabee, 1946). These two types of receptors interact in complex ways with each other and with central mechanisms. Reduced input from SARs and increased input from RARs during the deflation reflex would act to increase t_I and therefore phrenic height (Knowlton & Larrabee, 1946; Glogowska, Richardson, Widdicombe & Winning, 1972). Input

from RARs would also be expected to reduce t_E (Davies & Roumy, 1982) and to increase phrenic slope (Coleridge & Coleridge, 1986).

The effect of the loss of SARs (post SO_2) in the deflation reflex initiated by lung deflation with 10 cm H_2O is shown in Table 4.2. Variables (t_E , t_I , G, H) were calculated as percentage change from control breath. In the 1st breath, SO_2 reduced the deflation reflex. There was now no longer a large increase in t_I (pre SO_2 : $62.4 \pm 4.8\%$; post SO_2 : 6.9 ± 2.88) and H (pre SO_2 : 94.8 ± 6.97 ; post SO_2 : 13.52 ± 4.39), indicating the importance of SARs in affecting these two variables.

After SO_2 there is still a marked reduction in t_E of the 1st breath but in contrast to pre SO_2 there are now only small decreases in t_E in 2nd & 5th breaths. This suggests that SARs are very important in controlling t_E . With deflation, there will be a decrease in SARs activity which would cause shortening of t_E as SARs stimulation normally lengthens t_E . However the marked shortening of t_E of the 1st breath occurs after SO_2 could be caused by the marked increase in RARs activity which is caused by the initial deflation. Probably, as the receptors adapt very rapidly, this discharge will not continue to affect the 2nd & 5th breath.

The t_I of 2nd and 5th breath was increased after SO_2 , while it was not statistically changed before SO_2 . Phrenic H of the 2nd & 5th breath was increased after SO_2 as much as before SO_2 . These increases in t_I and phrenic H could be brought about by RARs and/or C-fibres activity induced in each breath. However, it is known that lung deflation is at best a weak stimulus to C-fibres (Paintal, 1969)

Table 4.2 The effect of SO₂ in deflation reflex (-10 cm H₂O) in normal rabbits. Variables were calculated as % change from control breath. Comparison was done by Wilcoxon Signed Rank Test. * p≤0.05, ** p ≤0.01, *** p≤0.0001.

-10 cm H ₂ O		1 st breath (Δ%)	2 nd breath(Δ%)	5 th breath(Δ%)
tE	Pre SO ₂	-27.3±2.9	-49.97±2.1	-40.3±3.01
	Post SO₂	-20.26±2.23	-7.95±2.14 ***	-4.69±1.72 ***
tI	Pre SO ₂	62.4±4.8	0.24±3.4	-4.4±2.6
	Post SO₂	6.91±2.88 ***	9.24±2.23 *	5.63±2.03 **
G	Pre SO ₂	12.97±0.96	15.65±0.79	15.78±1.5
	Post SO₂	6.14±1.13 ***	5.11±1.24 ***	6.92±1.74 **
H	Pre SO ₂	94.8±6.97	16.53±4.45	12.46±3.42
	Post SO₂	13.52±4.39 ***	15.76±3.59	14.36±3.71

Before administration of SO₂, deflation with 5 cmH₂O increased t_I and phrenic H of the 5th breath more in emphysematous rabbits than in normal animals. In response to -10 cmH₂O, t_I and phrenic H of the 2nd & 5th breaths were increased more in emphysematous rabbits than in normal rabbits (Table 3.23, Fig. 3.19). These effects might be mediated by more active RARs in emphysematous rabbits. RARs in a rat model of pulmonary emphysema were found to be more active (Pirie, 1997). It has been suggested that RARs are stimulated by atelectasis of the lung (Sant'Ambrogio, 1982), so therefore any form of deformation or collapse of the airways in emphysema may cause an increase in RARs activity.

After the administration of SO₂, deflation with 5 cmH₂O did not produce any differences in the measured parameters between the two groups (Table 3.25 & Fig. 3.22). However the stronger stimulus of a deflation with 10 cmH₂O increased t_I and phrenic H of 1st, 2nd & 5th breath more in emphysematous than in normal rabbits. These findings support the previous conclusion that RARs are more active in emphysematous than in normal rabbits. Deflation with 10 cmH₂O reduced the t_E of the 1st breath in normal (N) more than in emphysematous (E) rabbits (Table 3.25 & Fig. 3.22). This could be due to the fact that they were starting from different control values (N: 0.56 ±0.01; E: 0.46±0.02). It may also have reflected the importance of

the dynamic changes in airway resistance i.e. airway collapse in emphysematous animals during expiration, which would hinder shortening of t_E .

4.3.5 ACCELERATED BREATHING WITH CARBON DIOXIDE (CO₂)

The administering of a hypercapnic gas mixture to increase minute ventilation, is a frequently used test in experimental research. Changes in the pattern of breathing and in the drive to breathe observed in the present study during CO₂ increased minute ventilation, was used to simulate breathing during exercise, the time at which an emphysematous patient suffers the most disability due to dyspnoea (Guz, Noble, Eisele & Trenchard, 1970; Guz, 1977).

The effect of blockade of SARs (post SO₂) and bilateral vagotomy (postvagotomy) on the response to hypercapnia (6% CO₂) in normal rabbits is shown in Table 4.3. After SO₂ block hypercapnia reduced t_E and t_I and thus increased frequency as much as in pre SO₂. Intact RARs or/and C-fibres could be mediating this increase in frequency. Evidence from studies in rabbits (Russell, Raybould & Trenchard, 1984), where conduction in myelinated fibres (SARs and RARs) was blocked by anodal block, showed that the increase in f through a reduction in t_E and t_I to persist, indicating that this increase in f was mediated by C-fibres. In the present study although there was an increase in V_T in post SO₂, this increase was less than pre SO₂. That may be because post SO₂ V_T is 15% greater than pre SO₂. The increase in \dot{V}_I is less after SO₂.

After bilateral vagotomy in the present study, the decrease in t_E was significantly less than pre SO₂ and post SO₂, indicating the importance of RARs and/or C-fibres in mediating this effect. However Russell *et al.* (1984) showed that after SARs & RARs block by anodal block, rabbits still had a marked reduction in t_E . This suggests that C-fibres may be more important than RARs in decreasing t_E .

However, postvagotomy, t_I decreased as much as in pre and post SO₂, indicating that shortening of t_I in response to hypercapnia is independent of vagal afferent activity. Though Russel *et al.* (1984) reported an increase in t_I , their data were only presented qualitatively and statistical analysis was not performed. The increase in V_T postvagotomy, was less than pre SO₂. This might be because

postvagotomy V_T is 40% greater than pre SO_2 . \dot{V}_I postvagotomy was less than pre and post SO_2 .

Table 4.3 Effect of SARs block with SO_2 (post SO_2) and bilateral vagotomy (postvagotomy) in the response to 6% CO_2 in normal rabbits. Variables were calculated as % change from control breath. * # $p \leq 0.05$. * indicates significant difference between post SO_2 and postvagotomy vs. pre SO_2 . # indicates difference between post SO_2 and postvagotomy. Repeated Measure ANOVA on Ranks was used for comparison.

	Pre SO_2 ($\Delta\%$)	Post SO_2 ($\Delta\%$)	Postvagotomy ($\Delta\%$)
t_E	-21.7 \pm 2.6	-27.57 \pm 2.4	-3.3 \pm 1.04 * #
t_I	-15.9 \pm 1.3	-17 \pm 2.1	-10.06 \pm 2.25
f	24.9 \pm 2.7	30.9 \pm 3.3	7.1 \pm 1.17 * #
V_T	70.3 \pm 9.2	36.73 \pm 2.9 *	51.44 \pm 6.28 * #
\dot{V}_I	111.6 \pm 11.4	78.65 \pm 5.4 *	61.88 \pm 6.38 * #

To my knowledge, the response to CO_2 has never been measured in emphysematous animals. Before the administration of SO_2 , emphysematous and normal rabbits responded similarly to the breathing of 4% CO_2 , however, with 6% CO_2 , emphysematous rabbits reduced their t_I , and increased their f, \dot{V}_I and phrenic slope (G) more than normal rabbits (Table 3.27 & Fig. 3.24). This greater drive could have been mediated by RARs as it is known that their inputs are excitatory to breathing, and Pirie (1997) has shown that their activity is increased in emphysematous rabbits.

In agreement with the present study is the finding of Scano, Spinelli, Duranti, Gorinin, Gigliotti, Goti & Milic-Emili (1995). They studied the response of COPD patients to CO_2 . Comparing COPD patients without hypercapnia to control subjects, revealed that these patients have a greater minute ventilation and a greater rate of rise of electromyographic activity of the diaphragm ($p=0.06$), however, mouth occlusion pressure was not statistically different. In hypercapnic COPD patients there was a lower ventilatory response and this was attributed to mechanical impairment and inadequate chemoresponsiveness.

After SO_2 administration, emphysematous rabbits had a greater response to CO_2 administration than normal animals: a greater reduction in their t_i , greater increase in V_T , \dot{V}_I and phrenic slope (Table 3.29 & Fig. 3.26). This supports the earlier conclusion that RARs may be mediating this stronger drive to breathe.

After bilateral vagotomy, emphysematous rabbits had a larger response to the administration of 4 & 6% CO_2 : a higher V_T and \dot{V}_I and a greater phrenic slope than normal rabbits (Table 3.31 & Fig. 3.28), which may indicate the importance of extravagal inputs in emphysematous rabbits. There are several proposed sources for this drive, the first being chemical i.e. hypoxaemia; however both normal and emphysematous rabbits were given 100% O_2 to breathe to abolish this possibility. Both emphysematous and normal rabbits had similar end-tidal CO_2 values, which reflects arterial Pco_2 . They had normal arterial blood pressure, and their temperature was kept at constant level.

Inputs from respiratory muscle and chest wall could be implicated in this drive particularly at the higher level of V_T seen after bilateral vagotomy. Remmers & Marttila (1975) have demonstrated that afferent intercostal nerve stimulation can restore a reasonably normal respiratory rhythm in vagotomized animals. Emphysema is known to produce changes in the diaphragm and chest wall. Thomas, Supinski & Kelsen (1986) studied the changes in chest wall structure and elasticity in emphysematous hamsters; their data indicated that the resting position of the chest wall and its elastic properties are altered as a result of complex structural changes in the rib cage. It may be that these changes result in enhanced stimulatory discharge from muscle and rib cage receptors. Tamaoki (1988) studied the effect of elastase-induced emphysema on the histochemical properties of Guinea pig diaphragm and found that the number and size of oxidative fibres had increased. With this increased oxidative capacity, the animals may have a greater potential for ventilatory responses to increases in CO_2 .

Postvagotomy, normal rabbits show a slight decrease in t_E . However, the emphysematous rabbits were not able to shorten t_E , a difference that was statistically significant (Result: Table 3.31, Fig. 3.28). This could be explained by the fact that

emphysematous (0.77 ± 0.03) and normal rabbits (0.95 ± 0.02) were starting from different control levels. This inability may also reflect the importance of the dynamic changes in airway resistance i.e. airway collapse in emphysematous animals during expiration, which would hinder shortening of TE.

4.4 GENERAL DISCUSSION

Apart from the scientific interest in what is happening in lungs affected by the natural experiment of diseases like emphysema (an interest which provoked this study) there is the pragmatic interest in whether changes in breathing pattern, if any, in the disease, can be interpreted as changes, and in particular increases in drive to breathe. These may be associated with the sensation of dyspnoea, a symptom of which most emphysematous patients complain.

It is generally accepted that, at least in animals, activity from mechanoreceptors in the lungs affects the pattern of breathing. Experiments in animals showed that the pattern of vagally mediated volume information during inspiration determines both the shape and the duration of phrenic motoneurone output (Cross, Jones & Guz, 1980; Hwang & St.John, 1993). Various animal models of respiratory disease have implicated vagal afferents as the cause of the changed pattern of breathing. In a rabbit model of pulmonary fibrosis induced by intravenous injection of Oleic acid (Davies & Pack, 1991), and in a rat model of the same disease induced by bleomycin, vagal afferents have been shown to be responsible for the rapid, shallow breathing seen in these animals (Mansoor, Hyde & Schelegle, 1997b). In an experimental pneumonitis induced in dogs, Phillipson, Murphy, Kozar & Schultze (1975) concluded that vagal afferent stimuli were responsible for the excessive ventilation seen during exercise and this afferent information also contributed to the abnormal pattern of breathing. Vagal afferents have also been implicated in the genesis of increased frequency of breathing seen in rabbits with induced lung inflammation (Trenchard, Gardner & Guz, 1972)

The reflex response of pulmonary vagal receptors to changes in lung volume are similar in nature in man and animals (Guz & Trenchard, 1971b) although very different in potency. The inflation reflex is much weaker in man and may be modulated by the evolution of the power of speech. However, the importance of

vagally mediated inputs may be of more importance in disease conditions (Campbell & Guz, 1986). Newborn babies and unconscious adult man have a classical Hering-Breuer inflation reflex of the type displayed in animals (Cross, Klaus, Tooley & Weisser, 1960; Guz, Noble, Trenchard, Cochrane & Makey, 1964); and conscious man probably demonstrates a potent irritant reflex (Guz, Noble, Widdicombe, Trenchard & Mushin, 1966; Simonsson, Jacobs & Nadel, 1967). Furthermore, vagal blockade in man interferes with ventilatory response to CO₂ as it does in conscious animals (Guz *et al.*, 1966; Phillipson, Hickey, Bainton & Nadel, 1970). Consciousness may alter the strength of the inflation reflex, but it does not mean that inputs from receptors are blocked by consciousness.

It is also generally accepted that the activity of these receptors is affected by their physical and chemical surroundings. Measurements of lung mechanics and histopathology make it quite clear that in most diseases of the lung, its architecture and therefore physical properties are changed. The chemical and mechanical changes due to lung disease may lead to changes in the firing pattern and/or sensitivity of lung afferents (Davies & Pack, 1991; Davies & Pirie, 1995). This, in turn, may lead to changes in the reflexes initiated in the lung, changes in pattern of breathing and changes in the control of breathing which may express themselves clinically as altered sensation. Patients with pulmonary fibrosis have a characteristic rapid, shallow breathing, which has been attributed to activity in vagal afferents, although a role for consciousness and chest wall afferents was also proposed (Savoy, Dhingra & Anthonisen, 1981).

In frank cases of pulmonary emphysema, the lung looks like Swiss cheese, due to loss of tissue and it becomes excessively compliant. In the present study it has been shown that there are similar morphometric changes in the rat and rabbit models of emphysema (Result: page 109). In addition, it has been shown that in both models there is an increase in lung static compliance (Result: page 108).

Previous work from this laboratory (Pirie, 1997) has described activity in the vagus nerves of rats rendered emphysematous with papain, as changes in pattern of discharge of both slowly and rapidly adapting receptors. It was found that emphysematous rats had more active SARs and RARs than normal rats. SARs of

emphysematous rats were found to fire with greater peak frequency than those of normal rats, RARs of emphysematous rats had greater peak frequency, greater number of spikes per second and greater number of spikes per phase during expiration.

In the current investigation the role of the receptors in the pattern of breathing and respiratory drive was studied by investigating various manoeuvres, pre SO₂ with the vagal receptors intact, post SO₂ where the SARs were blocked and also postvagotomy. The main differences in the measured parameters between normal and emphysematous rabbits found in this study has been summarised in Tables 4.4, 4.5 & 4.6. In these Tables, double arrow signs are used to indicate significantly larger (↑↑) or smaller (↓↓) changes when normal and emphysematous rabbits are compared

Tables 4.4 shows that there are marked differences between normal and emphysematous rabbits in the control, anaesthetised state. Although t_E is longer and thus breathing frequency is less in emphysematous rabbits, nonetheless, because of the increase in V_T there is an increase in \dot{V}_I . This increased ventilation must be brought about by an increased drive to breathe, indeed phrenic slope (G) and phrenic height (H) were greater in emphysematous rabbits but that increase was not statistically significant. It can also be seen in Table 4.4 that after the removal of SARs with SO₂ there is a depression of ventilation and drive to breathe (reduction in phrenic G) in normal rabbits associated with lengthening of both t_E & t_I and thus a decreased frequency of respiration. In comparison, there was an increase in ventilation and drive to breathe in emphysematous rabbits. Therefore it seems that SARs are relatively less important in emphysematous rabbits than normal rabbits in maintaining ventilation and the drive to breathe. It can also be seen in Table 4.4 that after vagotomy there was much larger reduction in ventilation and drive to breathe in emphysematous than in normal rabbits. Therefore the RARs and/or C-fibres are probably relatively more important in emphysematous than in normal rabbits. In the present study no attempt has been made to separate the reflex effect of RARs and C-fibres. However previous studies in an animal model of pulmonary emphysema showed that C-fibre reflex activity was not changed in emphysematous animals (Delpierre *et al.*, 1985; Mansoor *et al.*, 1997)

The Hering-Breuer inflation reflex (HBIR) was found to be not significantly different between normal and emphysematous rabbits, although it was larger in normal rabbits, therefore, it is not included in the Tables 4.4, 4.5, 4.6. Even though, after SO₂, there were some small differences found between rabbits, these may not be physiologically significant as there was no significant differences observed between the two groups with 10 cm H₂O inflation.

In contrast to the lack of differences between the normal and emphysematous rabbits during inflation manoeuvres, there were marked differences observed during deflation procedures. It can be seen in Table 4.5 that deflation prior to SO₂ caused a much greater increase in *t_i* and phrenic H in emphysematous animals, which would probably lead to increased ventilation. These differences between the two groups were maintained after SO₂ administration. This indicates that SARs were not involved. Therefore, the changes in the measured variables must have been due to a greater responsiveness to deflation in emphysematous rabbits brought about by RARs and/or C-fibres. It is known that lung deflation is at best a weak stimulus to C-fibres (Paintal, 1969).

Emphysematous rabbits also had an enhanced response to CO₂ as is clearly shown in Table 4.6. They had greater response to 6% CO₂ before SO₂ (pre SO₂), after SO₂ (post SO₂) and after bilateral vagotomy (postvagotomy). This greater drive to breathe before and after SO₂ could have been mediated by RARs as it is known that their inputs are excitatory to breathing. The greater drive to breathe in emphysematous rabbits after vagotomy could indicate the importance of input coming from chest wall and the diaphragm as it is known that emphysema produces changes in diaphragm and chest wall in animal models (Discussion: page 204).

Thus, in the porcine pancreatic elastase rabbit model of emphysema there is strong evidence that the enhanced activity of the RARs is responsible for the increased drive to breathe.

It may be that the enhanced RARs activity is also responsible for dyspnoea experienced in human patients with emphysema. If RARs are responsible it may be possible to find a way to diminish their activity and thus relieve the distressing symptom of dyspnoea. Alternatively, methods should be evolved to reduce the drive

to breathe and thus alleviate dyspnoea. In fact one recent method of treatment of emphysema by lung volume reduction surgery was found to diminish dyspnoea by a reduction in the drive to breathe (Celli, Montes, Mendez & Stetz, 1997).

Table 4.4 Main differences between normal and emphysematous rabbits. Post SO_2 was calculated as % change from pre SO_2 , postvagotomy was calculated as % change from post SO_2 . – indicates no significance difference between normal and emphysematous rabbits in the control state, $\uparrow\uparrow$ indicates significant larger increase in one type of rabbit compared to the other, $\downarrow\downarrow$ indicates significant larger decrease in one type of rabbit compared to the other, \uparrow indicates a small increase, \downarrow indicates a small decrease, \leftrightarrow indicates no significance change within group.

Control state (pre SO_2)	Normal rabbits	Emphysematous rabbits
t_E		$\uparrow\uparrow$
t_I		–
f		$\downarrow\downarrow$
V_T		$\uparrow\uparrow$
\dot{V}_I		$\uparrow\uparrow$
G		–
H		–
Post SO_2 ($\Delta\%$)	Normal rabbits	Emphysematous rabbits
t_E	$\uparrow\uparrow$	\leftrightarrow
t_I	$\uparrow\uparrow$	\uparrow
f	$\downarrow\downarrow$	\downarrow
V_T	\uparrow	\uparrow
\dot{V}_I	\downarrow	\uparrow
G	\downarrow	\uparrow
H	\leftrightarrow	$\uparrow\uparrow$
Postvagotomy ($\Delta\%$)	Normal rabbits	Emphysematous rabbits
t_E	\uparrow	$\uparrow\uparrow$
t_I	\uparrow	$\uparrow\uparrow$
f	\downarrow	$\downarrow\downarrow$
V_T	\uparrow	\uparrow
\dot{V}_I	\leftrightarrow	$\downarrow\downarrow$
G	\leftrightarrow	$\downarrow\downarrow$
H	\uparrow	\leftrightarrow

Table 4.5 Main differences between normal and emphysematous rabbits in their response to deflation reflex. Responses were calculated as % change from control breath. ↑↑ indicates significant larger increase in one type of rabbit compared to the other, ↓↓ indicates significant larger decrease in one type of rabbit compared to the other, ↑ indicates a small increase, ↓ indicates a small decrease, ↔ indicates no significance change within group.

-5 cm H₂O (pre SO₂)	Normal rabbits	Emphysematous rabbits
t _I (5 th breath)	↔	↑↑
H (5 th breath)	↑	↑↑
-10 cm H₂O (pre SO₂)	Normal rabbits	Emphysematous rabbits
t _I (2 nd breath)	↔	↑↑
H (2 nd breath)	↑	↑↑
t _I (5 th breath)	↔	↑↑
H (5 th breath)	↑	↑↑
-10 cm H₂O (post SO₂)	Normal rabbits	Emphysematous rabbits
t _E (1 st breath)	↓↓	↔
t _I (1 st breath)	↑	↑↑
H (1 st breath)	↑	↑↑
t _I (2 nd breath)	↑	↑↑
H (2 nd breath)	↑	↑↑
t _I (5 th breath)	↑	↑↑
H (5 th breath)	↑	↑↑

Table 4.6 Main differences between normal and emphysematous rabbits in their response to 6% CO₂. Responses were calculated as % change from control breath. ↑↑ indicates significant larger increase in one type of rabbit compared to the other, ↓↓ indicates significant larger decrease in one type of rabbit compared to the other, ↑ indicates a small increase, ↓ indicates a small decrease, ↔ indicates no significance change within group.

6% CO ₂ pre SO ₂ (Δ%)	Normal rabbits	Emphysematous rabbits
t _i	↓	↓↓
f	↑	↑↑
\dot{V}_I	↑	↑↑
G	↑	↑↑
H	↔	↑
6% CO ₂ Post SO ₂ (Δ%)	Normal rabbits	Emphysematous rabbits
t _i	↓	↓↓
V _T	↑	↑↑
\dot{V}_I	↑	↑↑
G	↑	↑↑
H	↔	↔
6% CO ₂ Postvagotomy (Δ%)	Normal rabbits	Emphysematous rabbits
t _E	↓	↑
V _T	↑	↑↑
\dot{V}_I	↑	↑↑
G	↑	↑↑
H	↔	↑

Summary

1. The aim of this project was to investigate whether pulmonary emphysema changed respiratory drive, as it has been proposed that changed respiratory drive might be responsible for the sensation of dyspnoea experienced by many human patients with emphysema.
2. Both rat and rabbit models of emphysema were produced. The pattern of breathing was examined by studying t_E , t_I , V_T and \dot{V}_I . The respiratory drive was assessed by studying the slope (G) and the peak (H) of phrenic activity.
3. Emphysema was successfully produced as it was found to increase both mean linear intercept and static lung compliance.
4. Results of rats' conscious pattern of breathing and rabbits' anaesthetised pattern of breathing showed that emphysema increased t_E and V_T . The longer t_E was thought to be produced by more active SARs. The larger V_T may have reflected an increase drive to breathe.
5. The role of pulmonary receptors in changing the pattern of breathing and the respiratory drive was assessed by studying these in acute experiments. They were studied when all the pulmonary receptors were intact (pre SO_2), after SARs were blocked with SO_2 and after all vagal receptors were removed by bilateral vagotomy (postvagotomy).
6. The state of the SARs and RARs in this model was assessed by studying the strength of inflation and deflation reflexes.
7. Removal of SAR activity by SO_2 decreased ventilation and decreased the phrenic slope (G) in normal rabbits, while in emphysematous rabbits ventilation, G and phrenic height (H) were increased. After vagotomy there were much larger reductions in ventilation and in the phrenic slope (G) in emphysematous

than in normal rabbits. These results indicate that RARs and not SARs are more important in emphysematous than in normal rabbits.

8. The inflation reflex (HBIR) was not statistically different between the two groups of rabbits. However the deflation reflex was stronger in emphysematous than in normal rabbits, indicating that the RARs are more active in emphysematous rabbits.
9. The response to CO₂ indicated that emphysematous rabbits had a greater drive to breathe (as indicated by an increase in phrenic slope, G) and a greater ventilation than normal rabbits before any receptors were blocked (preSO₂), after blocking the SARs (postSO₂) and after removal of all vagal receptors inputs (postvagotomy).
10. In conclusion this study showed that emphysematous rabbits had a greater drive to breathe in the control state and in response to CO₂. If this increased drive to breathe is also a feature of emphysema in man, then this could be a contributing factor to the increased sensation of dyspnoea. The results also suggested that RARs are more active in emphysematous rabbits, and therefore they could be mediating the stronger drive to breathe. Therefore it may be possible to alleviate dyspnoea by reducing the drive to breathe either directly or through reducing RAR activity.

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APPENDIX 1

COMPUTER SCRIPT USED FOR ANALYSING DATA IN THE SPIKE 2 (CED) SOFTWARE PACKAGE

'Phrenic Nerve Analyser V3

'(c) Rich Software 1996

```
Var pchan fchan value nt newtime samptime userbins binb
Var store[2000] store2[2000]
Var average maximum minimum ubinsize loop hcur1 hcur3 commit
Var outfile$ oldfile$ err finish result cur teint tiint itotal
Var bstart bfinish pchange histog ohistog
Var mdtime mdtot mdstart
Var sclow schigh scrange sccentre
Var oldc1 oldc2 oldc3
Var tottmp
Var diff1 diff2
Var rndtmp
Var minbin mintime
Var actav
Var rgstart rgfinish rgxs rgsy rgxsx rgsxy rggrad rgint rgnum
```

'Draw screen and initialise global variables

histog:=0

ohistog:=0

setup

pchan:=2

fchan:=1

samptime:=binsz[pchan]

ubinsize:=0.1

hcur1:=0

hcur3:=0

outfile\$=""

repeat

repeat

'Put up the Analysis loop menu until we have sensible data

err:=1

oldfile\$:=outfile\$

menu 0 "Analysis Loop" 4

menu 1 1 "Output filename" 3 12

menu 1 2 "Bin size (s)" 1 0 100

menu 1 3 "Draw Histograms" 4 "No" "Yes"

menu 1 4 "Finished" 4 "No" "Yes"

menu 2 result outfile\$ ubinsize histog finish

if finish=1

'Close any output files and terminate


```

        printto ""
        end
    endif

    if oldfile$<>outfile$
        'Check to see if file is already present
        file outfile$ err
        if err=2 ; message "This file exists already" ; endif
    endif

until err=1

    if ohistog<>histog
        setup
        ohistog:=histog
    endif

'Analyse the phrenic activity
analyse

'Calculate VTe and VTi
calcint c1 c2
teint:=itotal
calcint c2 c3
tiint:=itotal

diff1:=c2-c1
diff2:=c3-c2
' Print out the integrals
print "c1-c2 %d-%d c2-c3 %d-%d" diff1 teint diff2 tiint
interact

'Calculate the minimum activity between c1 and c2
minact 0 binb

'Determine the normalised activity between c1 and c3
for loop:=0 userbins

```

```

    store2[loop]:=store[loop]-minimum
next loop

    if histog=1
'Draw the axes for the activity histogram
setupact

'Draw the absolute activity histogram
colour 13 3

drawact 0

'Draw the normalised activity histogram
colour 13 4

drawact 1
view 1
endif

print "Done."

query "Commit data to file?" "y or n then enter" "y" commit
if commit=1.0
    'Write the gathered data to the output file

```

```

if oldfile$<>outfile$
  'If the filename has changed, initialise the new file for output
  printto outfile$
endif

'Work out area of first strip and % change
bfinish:=c1
DoBin 0

actav:=0
for loop:=0 minbin
  actav:=actav+store[loop]
next loop
actav:=actav/(minbin+1)

mintime:=minbin*ubinsize
print -3 "%d,%d,%d,%d," store[0] store2[0] pchange itotal
print -3 "%d,%d,%d,%d,%d," c1 c2 c3 hcur1 hcur3
print -3 "%d,%d,%d,%d," ubinsize userbins+1 binb+1 minimum
print 3 "%d,%d,%d,%d" store[0] store[binb] mintime actav

'Work out area of second strip and % change
DoBin 1

print -3 "%d,%d,%d,%d," store[1] store2[1] pchange itotal
print 3 "%d,%d,%d,%d" c2-c1 c3-c2 teint tiint

mdstart:=binb+1
mdtime:=0
mdtot:=0

'Print out the rest of the bins
for loop:=2 binb
  DoBin loop
  print 3 "%d,%d,%d,%d" store[loop] store2[loop] pchange itotal
next loop

rgsx:=0
rgsy:=0
rgsxs:=0
rgsxy:=0

for loop:=mdstart userbins
  DoBin loop
  mdtime:=mdtime+ubinsize
  mdtot:=mdtot+pchange
  print -3 "%d,%d,%d,%d," store[loop] store2[loop] pchange itotal
  print 3 "%d,%d" mdtime mdtot

```

```

if loop<userbins
  rgxs:=rgsx+mdtime
  rgsy:=rgsy+mdtot
  rgxs:=rgxs+(mdtime*mdtime)
  rgxy:=rgxy+(mdtime*mdtot)
  rgfinish:=mdtot
endif
if loop=mdstart
  rgstart:=mdtot
endif
next loop

rgnum:=userbins-mdstart
rggrad:=((rgnum*rgxy)-(rgsx*rgsy))/((rgnum*rgxs)-(rgsx*rgsx))
rgint:=((rgxs*rgsy)-(rgxy*rgsx))/((rgnum*rgxs)-(rgsx*rgsx))

print 3 "Gradient,Y-Intercept,Start,Finish"
print 3 "%d,%d,%d,%d" rggrad rgint rgstart rgfinish
print 3 "----,----,----,----"

endif

  if histog=1
    'Allow the user to adjust the histogram until hitting ESCAPE
    drawfkey
    repeat
      fkey 5
    until escape
  endif
until 0
end

'Set up the function keys for Redraw
proc drawfkey
  fkey 0
  fkey 1 2 drawv2 "Redraw"
  return
end

'Set up the function keys for Cursor Movement
proc movefkey
  fkey 0
  fkey 1 3 movec1R "C1 >"
  fkey 1 4 movec2R "C2 >"
  fkey 1 5 movec3R "C3 >"
  fkey 1 6 zoomin "Chan1+"
  fkey 1 7 zoomout "Chan1-"
  fkey 1 8 zminph "Chan2+"

```

```

    fkey 1 9 zmoutph "Chan2-"
    return
end

```

```

proc zoomin
    scentre:=HC
    yrange 1 sclow schigh 1
    scrange:=(schigh-sclow)/4
    sclow:=(scentre-scrange)
    schigh:=(scentre+scrange)
    yrange 1 sclow schigh
    draw
    return
end

```

```

proc zoomout
    scentre:=HC
    yrange 1 sclow schigh 1
    scrange:=(schigh-sclow)
    sclow:=(scentre-scrange)
    schigh:=(scentre+scrange)
    yrange 1 sclow schigh
    draw
    return
end

```

```

proc zminph
    scentre:=HC
    yrange 2 sclow schigh 1
    scrange:=(schigh-sclow)/4
    sclow:=(scentre-scrange)
    schigh:=(scentre+scrange)
    yrange 2 sclow schigh
    draw
    return
end

```

```

proc zmoutph
    scentre:=HC
    yrange 2 sclow schigh 1
    scrange:=(schigh-sclow)
    sclow:=(scentre-scrange)
    schigh:=(scentre+scrange)
    yrange 2 sclow schigh
    draw
    return
end

```

'Move cursor 1 to the right until it is at the time that the
'flow curve next crosses the horizontal axis
proc movec1R

```
cur:=c1  
movecurR  
setc 1 cur  
return
```

end

'Move cursor 2 to the right until it is at the time that the
'flow curve next crosses the horizontal axis
proc movec2R

```
cur:=c2  
movecurR  
setc 2 cur  
return
```

end

'Move cursor 3 to the right until it is at the time that the
'flow curve next crosses the horizontal axis
proc movec3R

```
cur:=c3  
movecurR  
setc 3 cur  
return
```

end

'Find where the flow curve crosses the horizontal cursor
'Starts looking from time CUR, changes CUR to the time of the crossing
proc movecurR

Var oldtime newtime oldsamp newsamp found curvalue

```
'Make sure horizontal cursor is on the flow channel  
'and get the (possibly changed) Y value into hcur1  
'hcursor fchan  
hcur1:=HC
```

```
'Get the Y value of the flow channel at the nearest time to the cursor  
nexttime fchan cur oldtime curvalue
```

```

oldsamp:=curvalue
found:=0
while found=0
    'Get the next flow sample value
    nexttime fchan oldtime newtime newsamp
    if curvalue<hcur1
        'Curve is rising
        if newsamp>hcur1 ; found:=1 ; endif
    else
        'Curve is falling
        if newsamp<hcur1 ; found:=1 ; endif
    endif
    if found=0
        'Transition above/below horizontal cursor not yet found
        'Set up to look at the next sample value in the sequence
        oldsamp:=newsamp
        oldtime:=newtime
    endif
wend

'Transition found, calculate the X position of the cursor
'print "p %d %d n %d %d h %d c %d" oldsamp oldtime newsamp newtime hcur1
cur
'interact
cur:=abs((hcur1-oldsamp)/(newsamp-oldsamp))
cur:=cur*(newtime-oldtime)+oldtime
return
end

'Draw the views
proc drawv2

    clear
    view 2
    setupact
    colour 13 3
    drawact 0
    colour 13 4
    drawact 1
    view 1
    draw
    return

end

'Draw a marker at the given time and height in the given colour
proc plotat pos val col

```



```

colour 13 col

mover pchan pos-0.0025 val
drawr pchan pos+0.0025 val
mover pchan pos val-0.2
drawr pchan pos val+0.2
return
end

'Use the ValueAt function to plot points along the waveform between
'times start and finish, with the given step. Useful for debugging.
proc ploterv start finish step

var loop

colour 13 6

for loop:=start finish step
  valueat pchan loop
  mover pchan loop-0.0002 value
  drawr pchan loop+0.0002 value
  mover pchan loop value-0.2
  drawr pchan loop value+0.2
next loop
return
end

proc setupact

var minX maxX

'Get the X axis range for the data channels
view 1
minX:=screenl
maxX:=screenr

'Setup the X and Y axis range for the histogram
view 2
maxact 0 userbins
minact 0 userbins
yrange 1 -0.1 maximum+0.1
draw minX maxX
return

end

'Draw the given activity histogram
proc drawact arrnum

```

```

var oldlp lp loop yval

view 1
lp:=c1
view 2
loop:=0
repeat
    if arrnum=0
        yval:=store[loop]
    else
        yval:=store2[loop]
    endif
    mover 1 lp 0
    drawr 1 lp yval
    oldlp:=lp
    lp:=lp+ubinsize
    drawr 1 lp yval
    drawr 1 lp 0
    drawr 1 oldlp 0
    loop:=loop+1
until loop>userbins
return

end

'Get input in the form of cursor movements and function key
'activations until the escape key is pressed
proc getIP

    repeat
        fkey 5
    until escape
    return

end

'Calculate the integral of the flow channel between start and finish
proc calcint start finish

    var prevtime newtime sampval previval newival samptime

    'print "Calculating integral..."
    samptime:=binsz[fchan]
    itotal:=0
    prevtime:=start

    'Integral first sample value

```

```

valueat fchan prevtime
rndtmp:=abs(value-hcurl)
' rndtmp:=rndtmp*1000
' rndtmp:=round(rndtmp)
' rndtmp:=rndtmp/1000
previval:=rndtmp

repeat
  'Add the area of this strip to the total
  nexttime fchan prevtime newtime sampval
  rndtmp:=abs(sampval-hcurl)
  ' rndtmp:=rndtmp*1000
  ' rndtmp:=round(rndtmp)
  ' rndtmp:=rndtmp/1000
  newival:=rndtmp
  tottmp:=((0.5*(newival+previval))*(newtime-prevtime));
  itotal:=itotal+tottmp
  previval:=newival
  prevtime:=newtime
until (prevtime+samptime)>=finish

'Get last integral value
valueat fchan finish
rndtmp:=abs(value-hcurl)
' rndtmp:=rndtmp*1000
' rndtmp:=round(rndtmp)
' rndtmp:=rndtmp/1000
newival:=rndtmp
itotal:=itotal+((0.5*(newival+previval))*(finish-prevtime))

rndtmp:=itotal*1000
rndtmp:=round(rndtmp)
itotal:=rndtmp/1000

'print "Done."
return

end

'Analyse the phrenic activity
proc analyse

  var eventcnt numsamps prevtime newtime avval
  var sampval total samples endofbin

  view 1
  cursors 3
  hcursor fchan hcurl

```

```

print "Please set cursors on channel 1, then hit Escape"
'Delay so the user can read the message
repeat
until inkey<0
delay 2

'Get cursor/function key input
movefkey
getIP
hcur1:=HC

' Save cursors before changing channel
oldc1:=c1
oldc2:=c2
oldc3:=c3
'Put horizontal cursor on pchan
hcursor pchan hcur3

print "Please set cursors on channel 2, then hit Escape"
'Delay so the user can read the message
repeat
until inkey<0
delay 2

getIP
hcur3:=HC

'Return horizontal cursor to the flow channel at its original position
hcursor fchan hcur1

' Restore cursors
setc 1 oldc1
setc 2 oldc2
setc 3 oldc3

print "Processing..."
newtime:=c1
userbins:=-1
binb:=-1
repeat
    prevtime:=newtime
    endofbin:=newtime+ubinsize
    valueat pchan prevtime
    'plotat prevtime value 5
    total:=abs(value-hcur3)
    'total:=(value-hcur3)
    samples:=1

```

```

repeat
  nexttime pchan prevtime newtime sampval
  'plotat newtime sampval 6
  total:=total+abs(sampval-hcur3)
  'total:=total+(sampval-hcur3)
  samples:=samples+1
  prevtime:=newtime
until (prevtime+samptime)>=endofbin
valueat pchan endofbin
'plotat endofbin value 7
total:=total+abs(value-hcur3)
'total:=total+(value-hcur3)
'total:=abs(total)
samples:=samples+1

newtime:=endofbin
avval:=total/samples
userbins:=userbins+1

store[userbins]:=avval
if binb=-1
  if newtime>=c2
    binb:=userbins
  endif
endif
until newtime>=c3
return

end

'Calculate the average activity between bins start and finish
'Returns the calculated result in global variable AVERAGE
proc avact start finish

  var loop total

  total:=0
  for loop:=start finish
    total:=total+store[loop]
  next loop
  average:=total/(finish-start+1)
  return

end

'Calculate the maximum activity between bins start and finish
'Returns the calculated result in global variable MAXIMUM
proc maxact start finish

```

```

var loop

maximum:=0
for loop:=start finish
  if store[loop]>maximum
    maximum:=store[loop]
  endif
next loop
return

end

'Calculate the minimum activity between bins start and finish
'Returns the calculated result in global variable MINIMUM
proc minact start finish

  var loop

  minimum:=store[start]
  for loop:=start finish
    if store[loop]<minimum
      minimum:=store[loop]
      minbin:=loop
    endif
  next loop
  return

end

proc setup

  var channel minval maxval

  clear
  view 1
  on 1
  on 2
  for channel:=1 2
    minmax channel 0 maxtime minval maxval
    yrange channel minval maxval
    'print "C %d min %d max %d" channel minval maxval ; interact
  next channel
  'fkey 0
  'fkey 1 2 drawv2 "Redraw"
  view 1
  if histog=1
    window 0 0 100 70

```

```

else
    window 0 0 100 98
endif
if histog=1
    setaxes 2 ; view 2
    yrange 1 0 10
    view 2 ; window 0 70 100 98
endif
clear
if histog=1
    view 2
    draw 0 10
endif
view 1
draw 0 10
return
end

```

'Returns the waveform Y value at time sTime in global variable VALUE.

'Returns the time from which the next search should be made to get

'the sample at the time following sTime in global variable NEWTIME.

'Interpolates between waveform sample times if necessary.

```

proc ValueAt channel sTime

```

```

    Var sampval1 sampval2 sampval3 time1 time2 time3 time4 samptime

```

```

    'Get the sample time for the given channel

```

```

    samptime:=binsz[channel]

```

```

    'If sTime is at or past the last sample time then we return the

```

```

    'waveform value at maxtime and newtime is set to -1

```

```

    if sTime>=maxtime

```

```

        time1:=maxtime-0.5*samptime

```

```

        nexttime channel time1 newtime value

```

```

        newtime:=-1

```

```

        'print "sTime %d value %d newtime %d" sTime value newtime ; interact

```

```

        return

```

```

    endif

```

```

    'Get the waveform value at the sample time preceeding

```

```

    'sTime, and the value at the sample time that follows this one

```

```

    'time2 becomes the time at which the waveform is at sampval2

```

```

    'time3 becomes the time at which the waveform is at sampval3

```

```

    'despite what the documentation says!

```

```

    time1:=sTime-samptime

```

```

    nexttime channel time1 time2 sampval2

```

```

    nexttime channel time2 time3 sampval3

```



```

if sTime<=time2
    'sTime lies in the region from 0 seconds to the first sample
    'time (time2), so we arbitrarily return the waveform value at time2
    value:=sampval2
    newtime:=time2
else
    if time3=sTime
        'sTime is exactly a sample time, so return the exact value
        value:=sampval3
        newtime:=time3
    else
        'sTime lies between two sample times, so interpolate
        value:=(sTime-time2)/(time3-time2)
        value:=value*(sampval3-sampval2)+sampval2
        newtime:=time2
    endif
endif
endif
'print "time1 %d " time1 ; interact
'print "time2 %d sampval2 %d" time2 sampval2 ; interact
'print "time3 %d sampval3 %d" time3 sampval3 ; interact
'print "sTime %d value %d newtime %d" sTime value newtime ; interact
return
end

```

'Print out the data on the given channel

```

proc printchn chn

```

```

    var prevtime newtime value

```

```

    if chankind[chn]=1

```

```

        print "Sample time is %d" binsz[chn] ; interact

```

```

    else

```

```

        print "Channel %d is not a waveform channel!" chn ; interact

```

```

        return

```

```

    endif

```

```

    prevtime:=-1

```

```

    repeat

```

```

        nexttime chn prevtime newtime value

```

```

        print "prev %d new %d value %d" prevtime newtime value ; interact

```

```

        prevtime:=newtime

```

```

    until escape

```

```

end

```

'Work out the area of a bin and the percentage change

```

proc DoBin bin

```

```
bstart:=bfinish
bfinish:=bstart+ubinsize
calcint bstart bfinish
if store[bin]<>0
    pchange:=((store[bin]-minimum)/minimum)*100
else
    pchange:=99999
endif

return

end
```

APPENDIX 2



DEPARTMENT of PATHOLOGY

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DL/hb

17th December 1996

Mr M Dallak,
Dept of Physiology,
University Medical School.

Dear Mr Dallak,

You have asked me to comment on your experimental rabbit lungs with the regard to the pattern of emphysema produced by your experiments. You have given me four slides, two controls (C16RL and C14RM) and two experimental animals (24RM and D28LL). I have looked at these H&E sections subjectively without making any measurements.

Your control lungs look in good condition without any evidence of endemic infection. There is no apparent emphysema in these sections.

Your experimental lungs however do show what appears to be patchily distributed emphysema. It is difficult to give this a classification because I would not expect emphysema induced by intratracheal instillation of enzyme to match the anatomical types of emphysema seen in the human situation. In your lung some areas of the lung appear normal and there are variable degrees of emphysema which, though focally distributed, is probably patchy panacinar emphysema. The airways appear unaffected apart from the presence of some lymphoid infiltrate in one case. You have certainly produced a significant emphysema but I have not quantitated it.

Your histological blocks are, I assume, from lungs which have been handled, ie fixed by inflation in a comparable manner, and you have told me that you take standard size blocks. There is some variation in block size and it may be that you should control for this, though if the degree of processing and sectioning artefact is similar in the control and experimental animals this will not affect your results.

Yours sincerely,

Dr. D Lamb
BSc, MRCP, MB BS, PhD, FRCPath, FRCPEd.

PUBLICATIONS

Dallak, M., Xiujie Luan, Brown, H., Pirie, L. 1995. Stability of breathing patterns in men, rats and rabbits. *Journal of physiology* 483p.

Dallak, M., Davies, A., Moores, C. 1996. Pattern of breathing in a rabbit and a rat model of emphysema. *Journal of Physiology* 495p.

Davies, A., Dallak, M., Moores, C. 1996. Oral endotracheal intubation of rabbits (*Oryctolagus cuniiculus*). *Laboratory animals* 30:182-183.

Stability of breathing patterns in men, rats and rabbits

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We intend to compare breathing pattern in conscious emphysematous and normal rabbits. To estimate the minimum number of emphysematous rabbits required to give a statistically valid result we need to know the variability of the pattern of breathing in normal rabbits.

We can find no record of such a measurement in the literature, although rabbits are said to have a highly labile pattern of breathing. The reproducibility of pulmonary mechanics has been measured in cattle (Galivan & McDonell, 1988) and breathing pattern of human beings has been measured in single instances (Tobin *et al.* 1983) and over time (Benchetrit *et al.* 1989).

We have adapted the whole body plethysmography method of Bartlett & Tenney (1970) developed from the method of Drorbaugh & Fenn (1955) to measure the breathing pattern of four rabbits and four rats on four separate days. The plethysmograph consists of a chamber through which a stream of air enters from a pump and leaves via a narrow tube. This tube offers high impedance to flow at the frequency of breathing of rats and rabbits. The breathing of the occupant of the chamber is therefore accurately reflected by small changes in pressure in the chamber. To precisely measure tidal volume the inlet and outlet of the chamber was closed for a few seconds. The breathing pattern of four human subjects was measured on four separate days, using an ultrasonic pneumotachograph (FIP Instruments, Field Road, Huntingdon, Cambridge). One hundred consecutive breaths were measured in terms of their inspiratory duration (t_i) expiratory duration (t_e) and tidal volume (V_T).

Variability of pattern was calculated as components of variance using the commercial analysis program SAS (SAS Institute Inc., SAS Circle, Box 8000 Cary, NC 27512-8000).

We calculate that to detect with 80% certainty a 10% change in the mean values of inspiration, expiration and tidal volume with the experimental protocol and species we have used, would require twelve men, nineteen rabbits, or eight rats respectively.

Supported by the Norman Salvesen Emphysema Research Trust.

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Pattern of breathing in a rabbit and a rat model of emphysema

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The pattern of breathing parameters (T_i , T_e , V_T)* of conscious control and emphysematous Dutch rabbits and rats was measured using the whole body plethysmography method of Bartlett & Tenney (1970), which was developed from the method of Drorbaugh & Fenn (1955).

Our previous study (Dallak *et al.* 1995) showed that to detect a 10% change in the breathing pattern parameters at 80% power, it is necessary to measure twenty breaths per day for 4 days. The number of Dutch rabbits required to detect a 10% change in T_i , T_e and V_T is 10, 19 and 3 and that of rats is 4, 8 and 6.

Emphysema was induced in six anaesthetized Dutch rabbits (under Hypnorm 0.4 ml kg⁻¹ i.m., Diazepam 1 mg kg⁻¹ i.v. and Butorphanol 0.1 mg kg⁻¹ i.v. as a reversal agent) by giving type 4 pancreatic elastase (240 units kg⁻¹ in 1 ml sterile 0.9% saline) by insufflation and in ten rats (under 2% Halothane anaesthesia) using papain (120 mg kg⁻¹ in 0.25 ml 0.9% saline). The pattern of breathing of the rabbits was measured before induction of emphysema and 4 weeks after, and that of the rats was measured before and 2 weeks after induction. The pattern of breathing parameters (means \pm s.d.) before and after induction of emphysema was as follows:

In rabbits: T_i , 0.54 ± 0.24 , 0.42 ± 0.11 , n.s.; T_e , 0.67 ± 0.20 , 0.50 ± 0.13 , n.s.; V_T , 13.3 ± 2.9 , 12.6 ± 3.1 , n.s.

In rats: T_i , 0.21 ± 0.02 , 0.22 ± 0.02 , n.s.; T_e , 0.31 ± 0.06 , 0.40 ± 0.07 †; V_T , 2.02 ± 0.30 , 2.45 ± 0.30 † († $P < 0.01$, Wilcoxon signed-rank test).

The results show that the induction of emphysema in the rabbits did not significantly alter the pattern of breathing parameters, while the induction of emphysema in rats made breathing deeper and slower.

Thus changes in the parameters describing the pattern of breathing following induction of emphysema are more readily detected in the papain rat model than in the elastase rabbit model.

* T_i is inspiration time in seconds, T_e is expiration time in seconds and V_T is tidal volume in millilitres.

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Oral endotracheal intubation of rabbits (*Oryctolagus cuniculus*)

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Summary

Endotracheal intubation of rabbits is reported, both personally and in the literature, to be so difficult that special equipment has been constructed by other workers to facilitate the procedure. We report that the positioning of the operator, behind the animal, viewing from the dorsal surface of the head, facilitates this procedure enormously.

Keywords Endotracheal intubation; rabbits; *Oryctolagus cuniculus*

To begin a study involving insufflation of drugs into the lungs of anaesthetized rabbits we required a method of endotracheal intubation which was quick, not traumatic to the rabbit's larynx and airway and which could be performed with certainty. All these requirements were directed to causing the least possible physiological stress and distress to the rabbits thus improving their welfare.

Tracheal intubation of the rabbit has been reported to us personally, and in the literature (Davis & Malinin 1974, Hoge *et al.* 1969, Schuyt & Leene 1977) as being difficult because of the mobility and anatomy of the larynx and upper airways. Special equipment has been designed to facilitate intubation (Schuyt *et al.* 1978) and a method relying on breath-sounds has been described (Alexander & Clark 1980). Ingenious methods, such as that of Kruger, Zellar & Schottmann (1994) demonstrate their considerable skill. We report here that the positioning of the operator is the most important determinant of success, and our finding that with correct positioning a difficult procedure became quick, easy and reliable.

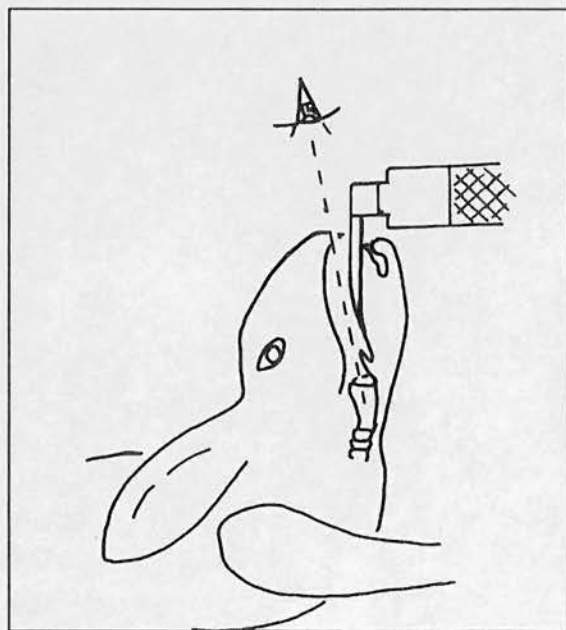


Fig 1 Visualization of the vocal cords

Materials and methods

Intubations have been performed on New Zealand White rabbits weighing 2.0–2.5 kg. They were anaesthetized via the marginal ear vein with Propofol (Zeneca) to a sufficient depth to allow the mouth to be easily opened.

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The rabbit was laid prone on a table with its head close to a corner and its body extended close to one edge of the table; so operator and assistant stood facing each other with the assistant in front of the rabbit and the operator behind and to one side of the animal. The rabbit's head was tipped back and supported at the angle of the jaw by the assistant who at the same time gently pulled the tongue out of one side of the mouth.

With the operator standing *behind* the rabbit a Wisconsin laryngoscope with paediatric blade Number 1 was inserted and by viewing from behind the dorsal surface of the head the vocal cords could be very well visualized (Fig 1).

A sterile endotracheal tube O.D.3.5 mm (Portex Ltd, Hythe, Kent CT21 6JL) lubricated with a water-soluble sterile lubricant (K-Y Jelly, Johnson & Johnson Ltd, Slough, UK) could easily be inserted from this position, even without the necessity of completely obtunding laryngeal reflexes. After insufflation of drugs the tube was withdrawn. Recovery was rapid and within 5 min all rabbits had regained their righting reflexes and were moving calmly about their cages. We have experienced no complications arising from this method of intubation.

Discussion

We have previously experienced considerable difficulties intubating rabbits. We understand this is a common experience. We recommend the method described as completely reliable, without trauma to the rabbits and without subsequent adverse effects.

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